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THE TAXONOMY OF *SMELOWSKIA CALYCINA* (STEPHAN) C.A. MEYER (CRUCIFERAE) IN NORTH AMERICA

by

© CRAIG W. GREENE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

FALL, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Taxonomy of *Smelowskia calycina* (Stephan) C.A. Meyer (Cruciferae) in North America" submitted by Craig W. Greene in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Cytological investigation has revealed a disparity between the chromosome numbers of southern (2n=12) and northern (2n=22) populations of *Smelowskia calycina* (Stephan) C. A. Meyer in the mountains of western North America. A review is given of the species' taxonomic position, synonymy and phytogeographical importance. A significant difference in cell size between diploids and tetraploids was found, but no other differences correlated with ploidy level were observed. Comparative investigations of morphology, leaf flavonoids and seed proteins show a low degree of similarity between populations of both diploids and tetraploids; it is suggested that the observed pattern of variation is indicative of the history of geographical isolation between populations of both chromosome races. *Smelowskia calycina* appears to be out-breeding and the cytological and reproductive behavior of the tetraploid is normal, with no form of apomixis in evidence. The range of the diploids is in the southern Rocky Mountains from Colorado, Utah and Nevada north to Washington and northwestern Montana. Tetraploids occur from northwestern Montana north to west-central Alberta and in Alaska, Yukon and the Northwest Territories. The establishment of allopatric ranges of each chromosome race is discussed in relation to the glacial history of North America and Asia during the Pleistocene. The autoploid derivation of the tetraploid during the Pleistocene is suggested, and the species' infraspecific classification is revised to include all tetraploid

populations in North America in subsp. *integrifolia* (Seeman) Hultén. The name subsp. *calycina* var. *montana* var. nov. is proposed for diploid populations in the southern Rocky Mountains.

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CHAPTER 1

INTRODUCTION

The genus *Smelowskia*[1] C.A. Meyer of the Cruciferae B. Juss, was published in 1831 to include three taxa previously ascribed to either *Lepidium* L. or *Hutchinsia* R. Br., but separable from them by characters of the fruit which show that the genus is better placed in the tribe Sisymbrieae DC. than in the tribe Lepidineae DC.[2] *Smelowskia* is a genus whose species are especially adapted to slide-rock and exposed gravel ridge habitats above timberline and whose distribution is restricted to mountainous central Asia, Siberia and the North American Cordillera (Map 1).

In his treatment of *Smelowskia*, Schulz (1924) recognized two sections, *Eusmelowskia* and *Polyctenium;* included in the latter section was *S. fremontii* Watson, a taxon Watson himself referred to the genus with some reservation[3]. Greene (1912a), citing characters of the fruit, removed this taxon from *Smelowskia* and named the genus *Polyctenium* to accommodate it. In his monograph of the North American

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[1] named in honor of Timotheus Smelowsky (1770-1815), a Russian botanist.

[2] Meyer (1831), in placing *Smelowskia* in the tribe Sisymbrieae, stressed the orientation of the seed in relation to the septum. In *Smelowskia,* the cotyledon margins lie against the septum, while in the tribe Lepidineae the cotyledons lie parallel to the septum with their margins facing the placentae.

[3] In describing *Smelowskia* (?) *fremontii*, Watson (1875) stated that "it much resembles *S. calycina* in habit but the characters of the fruit do not fully accord with those of the genus."

Map 1. Distribution of the genus *Smelowskia* C.A. Meyer

species of *Smelowskia* and *Polyctenium*, Rollins (1938) clearly showed the distinctness of the two genera based on habit and characters of the leaves and fruit.

Melanidion Greene was first described as a mono-typic genus of Alaska and Yukon with unknown generic affinities (Greene, 1912b). Hultén (1940) suggested its possible relationship to *Smelowskia*, but later concluded that *Melanidion boreale* Greene belongs in the genus *Ermania* Cham. (Hultén, 1945). The discovery of a new taxon (*Smelowskia pyriformis* Drury & Rollins) in central Alaska intermediate between *Smelowskia* and *Melanidion* led to the clarification of the affinities of the latter genus. Accordingly, Drury and Rollins (1952) have included *Melanidion boreale* Greene as *Smelowskia borealis* (Greene) Drury & Rollins in their monographic revision of *Smelowskia* in North America.

A description of *Smelowskia* (inclusive of *Melanidion* and exclusive of *Polyctenium*) follows:

> *Smelowskia* C.A. Meyer in Ledeb. Fl. Alt. 3:165. 1831. Caespitose perennial herbs with simple or branched caudex clothed with old leaf-bases; root long, woody; stems one to several, simple or branched, erect; plants glabrous to more often densely covered with a long villous pubescence of branched and simple trichomes; basal leaves petiolate, pinnate or bipinnately lobed, less often entire or few-lobed at the tip; cauline leaves few, as the basal leaves but reduced; inflorescence ebracteate except sometimes the lowest few flowers, corymbiform but becoming racemose in fruit; clayx segments equal, caducous or persistent; petals longer than the calyx, white or cream-colored or lavender, clawed, caducous; stamens tetradynamous; ovary sessile, style short but conspicuous; stigma usually entire, discoid, often expanded in fruit; fruit ovate or obovate to linear, sometimes inflated, the valves nerve-less to mostly prominently 1-nerved, mostly keeled, deciduous; seeds 2-18, uniseriate, wingless, not mucilaginous when wet; cotyledons incumbent. (Meyer, 1831; Busch, 1939; Drury and Rollins, 1952).

Smelowskia is represented by four species restricted to North America and three species restricted to central Asia and Siberia; only one species, *S. calycina*, is present on both continents (Table 1) and is of special interest in this study.

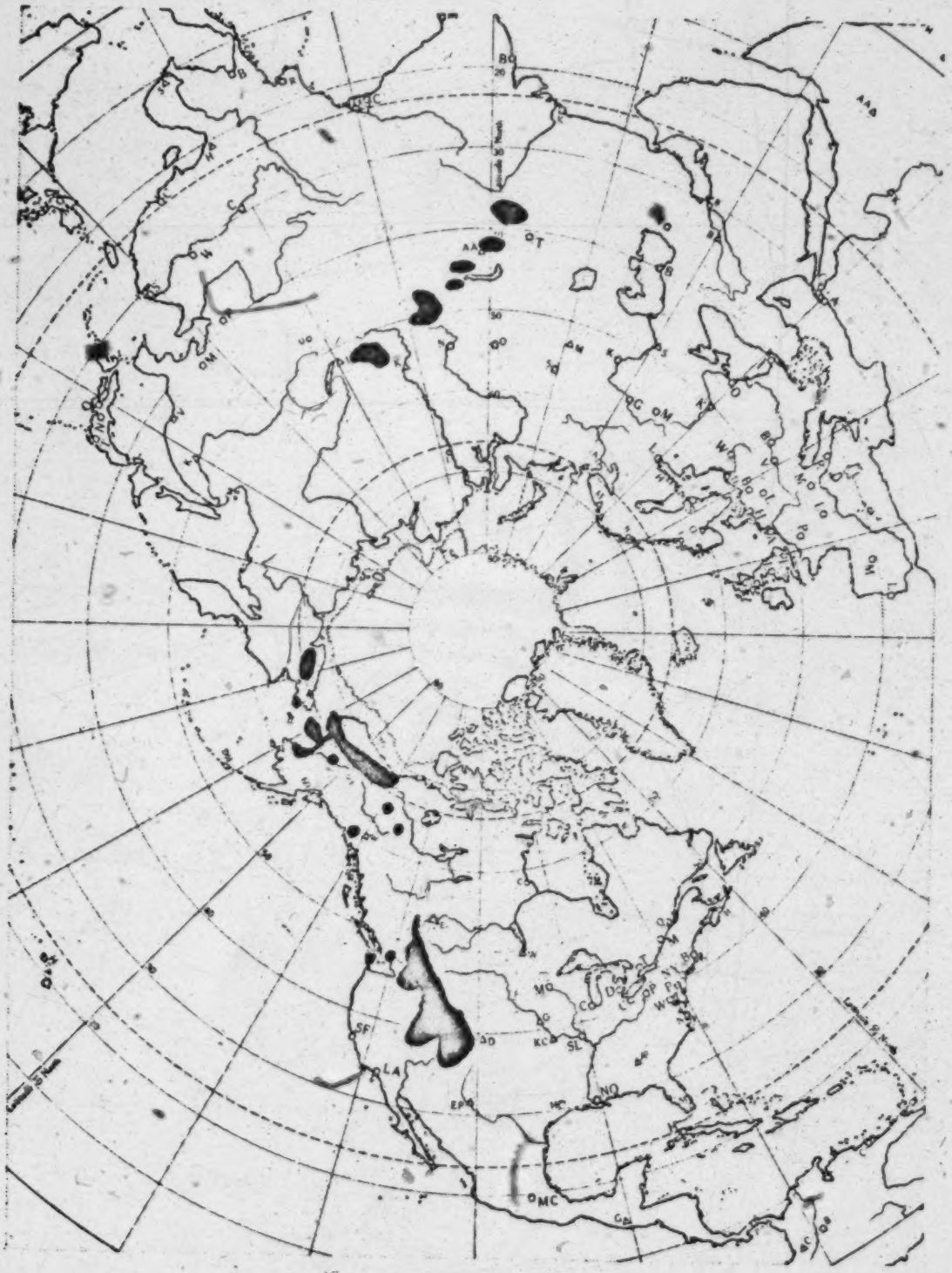
The geographical distribution of the genus (Map 1) suggests a once continuous arctic-alpine distribution from the Rocky Mountains north to Alaska and west to central Asia; this former distribution has been reduced to three main areas of persistence, in the mountains of central Asia and both north and south of the continental ice sheet in western North America. Of the species of *Smelowskia*, only *S. calycina* is present in all three regions (Map 2). The occurrence of the genus only within unglaciated or weakly glaciated areas implies a survival in these localities throughout the Pleistocene with no reinvasion of its former range subsequent to the retreat of the ice (Hultén, 1937). Drury and Rollins (1952, p. 95) even suggest that "it is possible that the mere existence of a population of the genus indicates an area of persistence."

Regarding the center of dispersal of *Smelowskia*, Drury and Rollins (1952, p. 96) propose an origin in the northern Cordillera region of North America, based on the presence of a higher number of species of the genus in North America than in Asia. However, because the persistence or extermination of these populations appears to be so dependent upon historical factors associated with the Pleistocene glaciations, it seems unwise to accept this contention, especially in the absence of any additional evidence.

There is a broad range of morphological variation among populations of most species of *Smelowskia*. This is indicative of the

Table 1. The species of *Smelowskia* and their Distribution according to Busch (1939) and Drury and Rollins (1952)

S. alba (Pall.) Regel	Siberia
S. asplenifolia Turcz	Siberia; cent. Asia
S. inopinata Kom.	Manchuria
S. calycina (Stephan) C.A. Meyer	Siberia; cent. Asia; Alaska; Yukon; Rocky Mts.
S. borealis (Greene) Drury & Rollins	Alaska; Yukon
S. pyriformis Drury & Rollins	Alaska
S. ovalis Jones	Wash.; Ore.; Calif.
S. holmgrenii Rollins	Nevada



Map 2. Distribution of *Smelowskia calycina*

survival of small populations in isolated mountain refugia resulting in an accentuation of the genetic variation present in widely separated parts of each species' range. (Hultén, 1937, p. 97). This allows the recognition of distinct geographical races in several species of the genus. Accordingly, Drury and Rollins (1952, pp. 97-106) have recognized four varieties among North American populations of *S. calycina*, four varieties of *S. borealis* and two of *S. ovalis*.

The only biosystematic studies of *Smelowskia* have been cytological examinations by a few workers; their published chromosome number reports are summarized in Table 2. These data show that the basic haploid chromosome complement of *Smelowskia* is n=6. The presence of both diploid (2n=12) and tetraploid (2n=22)[4] chromosome races of *S. calycina* in North America warrants further examination.

To date, the most thorough investigation of *Smelowskia calycina* and its infraspecific taxa in North America has been that of Drury and Rollins (1952). They recognize three varieties from Alaska and Yukon (var. *integrifolia* (Seeman) Rollins from the Seward Peninsula area, var. *porsildii* Drury & Rollins from the Brooks Range and var. *media* from the Richardson and British Mountains). The fourth variety, var. *americana* (Regel & Herder) Drury & Rollins, is widespread on the higher peaks of the Rocky Mountains from Colorado, Utah and Nevada north to Montana and Washington, but is much less abundant in the northern extension of its range into west-central Alberta.

In the synonymy of *S. calycina* and its varieties that follows

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[4]Although, strictly speaking, this represents an aneuploid-chromosome race, it is assumed to have been secondarily derived from a true tetraploid (2n=24) ancestor.

Table 2. Published Chromosome Counts of Species of *Smelowskia*

Taxon	2n=	Locality	Reference
S. alba (Pall.) Regel	12	Ulakhan-Alyn, N. Yakutskaya	Yurtsev & Zhukova (1972)
S. borealis (Greene) Drury & Rollins var. *villosa* Drury & Rollins	12	Mt. McKinley Park, Alaska	Drury & Rollins (1952)
S. calycina (Stephan) C.A. Meyer	12	Checkekty, Pamir	Yurtsev & Zhukova (1972)
S. calycina (Stephan) C.A. Meyer var. *americana* (Regel & Herder) Drury & Rollins	12	Hoosier Ridge, Summit Co., Colo.	Drury & Rollins (1952)
" " " "	12	" " " " "	Johnson & Packer (1968)
" " " "	12	Tushar Mt., Piutte Co., Utah	Drury & Rollins (1952)
" " " "	12	Clay Butte, Park Co., Wyo.	" " " "
	22	Mountain Park, Alberta	Packer (1968)
S. calycina (Stephan) C.A. Meyer var. *integrifolia* (Seeman) Rollins	22	Ogotoruk Creek, N.W. Alaska	Johnson & Packer (1968)
S. porsildii (Drury & Rollins) Yurtsev	24	Elgygytryn L., W. Chukotskiy	Yurtsev & Zhukova (1972)
" " " "	24	Amguema R., E. Chukotskiy	" " " "

the description of the species, names are referenced to those used by Drury and Rollins (1952).

Smelowskia calycina (Stephan) C.A. Meyer, in Ledeb. Fl. Alt. 3:170. 1831.
Densely caespitose alpine perennial of scree slopes and gravel ridges; taproot long, woody, surmounted by a branching caudex covered with persistent leaf bases; stems 1-several, unbranched, 5-20 cm high, sparsely to densely pubescent with short branched and long simple or branched trichomes; basal leaves petiolate, obovate to linear, 5-25 mm long, 3-10 mm wide, entire or pinnately 3-15 lobed, pubescence as on the stem; petioles conspicuously ciliate with long simple trichomes; cauline leaves 1-9, 3-20 mm long, similar to the basal leaves but reduced and nearly sessile; inflorescence corymbiform at beginning of flowering but becoming racemose in fruit; pedicels ascending to widely divaricate, 5-10 mm long, pubescent with long simple trichomes; calyx persistent or deciduous; sepals oval or oblong, 2.5-4.5 mm long, 1-2 mm wide, pubescent with long simple trichomes; petals white or cream-colored to rarely purplish, clawed, 3.5-7 mm long, 2-4 mm wide; siliques linear to oblong, 5-10 mm long, 1.5-2.5 mm wide, tapering at both ends, terete or slightly flattened parallel to the septum; valves strongly 1-nerved; septum complete or occasionally perforate; style 0.3-1.5 mm long; stigma expanded in fruit; seeds 2-10, ellipsoidal, 2 mm long, pendulous; cotyledons incumbent. (Busch, 1939; Drury and Rollins, 1952).

Synonymy of *Smelowskia calycina* and varieties[5]

Smelowskia calycina (Stephan) C.A. Meyer in Ledeb. Fl. Alt. 3:170. 1831.

Lepidium alpinum Sievers in Pallas, N. Nord. Beitr. 7:350. 1796. *Hom. illeg. non* Linnaeus 1756.

Lepidium calycinum Stephan, in Willd. Spec. Pl. 3(1):433. 1801.

Hutchinsia calycina (Stephan?) Desv., Journ. Bot. 3(4):168. 1814.

Bunias altaica Schangin ex Fisch. in DC. Syst. 2:388. 1821.

Smelowskia calycina var. *americana* (Regel & Herder) Drury & Rollins, Rhodora 54:99. 1952.

Hutchinsia calycina β *americana* Regel & Herder, Bull. Soc. Nat. Mosc. 39(2):101. 1866.

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[5]based on the synonyms given by Busch (1939, pp. 355-357) and Drury & Rollins (1952, pp. 98-102)

S. americana Rydb., Bull. Torr. Bot. Club 29:239. 1902. (*non β americana* Regel & Herder).

S. lineariloba Rydb., Bull. Torr. Bot. Club 31:555. 1904.

S. lobata Rydb., Bull. Torr. Bot. Club 39:327. 1912 (*pro parte*).

S. lineariloba f. *virescens* O.E. Schulz in Engler, Pflanzenr. 4(105):357. 1924.

S. calycina prol. *americana* O.E. Schultz in Engler, Pflanzenr. 4(105):356. 1924. *Nom. illeg.*

Smelowskia calycina var. *integrifolia* (Seeman) Rollins, Rhodora 40:300. 1938.

Hutchinsia calycina var. β Hook. Fl. Bor.-Am. 1:59. 1830.

H. calycina var. *integrifolia* Seeman, Bot. Voy. Herald: 25. 1852. (*non S. integrifolia* C.A. Meyer).

H. calycina var. γ Ledeb. Fl. Ross. 1:201. 1842.

S. calycina prol. *americana* f. *integrifolia* O.E. Schulz, in Engler, Pflanzenr. 4(105):356. 1924. *Nom. illeg.*

S. calycina subsp. *integrifolia* (Seeman) Hultén, Lunds Univ. Arssk. 41(5)869. 1945.

Smelowskia calycina var. *media* Drury & Rollins, Rhodora 54:100. 1952.

S. calycina subsp. *calycina* var. *media* (Drury & Rollins) Hultén, Arkiv för Bot. 7:64. 1968.

Smelowskia calycina var. *porsildii* Drury & Rollins, Rhodora 54:105. 1952.

S. calycina subsp. *integrifolia* var. *porsildii* (Drury & Rollins) Hultén, Arkiv för Bot. 7:64. 1968.

S. porsildii (Drury & Rollins) Yurtsev, Acad. Sci. U.S.S.R. 6:310. 1969.

These varieties were erected largely on the basis of leaf lobation and the degree of divergence of the pedicels; intergradations between them do occur. The named varieties, however, serve as useful points of reference in treating the broad range of morphological variation, especially among the populations of Alaska and Yukon. The

following key to the varieties of *S. calycina* incorporates most of the characters in which the varieties differ from one another.

Key to the presently recognized varieties of *Smelowskia calycina* in North America[a]

Basal leaf-blades entire or shallowly lobed at the tips; cauline leaves entire or shallowly 3-lobed.
- Basal leaf-blades linear to narrowly spatulate, shorter than the petioles, entire or shallowly few-lobed at the tip; pedicels in fruit divaricately ascending, the angle of divergence less than 60°; plants of the Brooks Range and interior northwestern Alaska ..... var. *porsildii*
- Basal leaf-blades obovate to oval, longer than the petioles, entire or with a few rounded lobes at the tip; pedicels in fruit widely divaricate, the angle of divergence 60°-90°; plants of the Seward Peninsula and western Brooks Range ..... var. *integrifolia*

Basal leaf-blades pinnately lobed or some of them entire; cauline leaves pinnately lobed.
- Pedicels widely divaricate in fruit, the angle of divergence 50°-80°; some basal leaf-blades usually entire; plants of the Richardson Mts. and the Mackenzie Range ..... var. *media*
- Pedicels weakly divaricate in fruit, the angle of divergence less than 50°; basal leaf-blades usually many-lobed, occasionally entire; plants of the southern and central Rocky Mts.... var. *americana*

[a]based on Drury and Rollins, 1952, p. 98.

Although this varietal classification provides a convenient system for treating the morphological variation within the species, the cytological evidence now available sheds doubt on its phylogenetic naturalness. This is apparent in the presence of both diploid and tetraploid populations within the taxon var. *americana*. In addition, var. *media*, considered by Drury and Rollins (1952, p. 91) to be the phylogenetic link between var. *americana* of the southern Rocky Mountains and var. *calycina* of Asia, has been cytologically examined

by this author and proven to be a tetraploid with 2n=22 chromosomes. Considering the largely irreversible nature of the processes involved in the formation of polyploids, the phylogenetic position of var. *media* must be re-evaluated.

Smelowskia calycina was cited by Hultén (1937, p. 96) as belonging to a group of species now possessing a much reduced and dissected distribution which, before the maximum advance of the ice during the Pleistocene, extended south of the maximum glaciation in an arctic-montane pattern in both North America and Asia. The present reduced distribution of the species (Map 2) shows its occurrence throughout the range of the genus. Hultén's conclusions about the history of the species were based on the assumption that this distribution represents that of a single biological unit. If, however, the distribution of tetraploid populations is widespread, it will be necessary to revise Hultén's analysis to account for the origin and migration of both tetraploid and diploid populations to their present ranges.

The present study was initiated to determine the distributions of the chromosome races of *S. calycina* in North America and to examine their relationship to one another, using morphological and phytochemical criteria. This information allows a re-evaluation of both the migrational history of the species during the Pleistocene as proposed by Hultén (1937) and its infra-specific classification as advanced by Drury and Rollins (1952).

Also of interest in the present investigation is the distribution of *S. calycina* in the mountains of Alberta; populations of the species grow in several isolated mountain localities in the

province as far north as the 53rd parallel (in the vicinity of Mountain Park, Alberta), well north of the supposed southern limit of Pleistocene glaciation in the mountains (Packer and Vitt, 1974). As has been previously mentioned, *S. calycina* seems to be an indicator of refugial areas, and its presence in such northern localities seems indicative of persistence throughout the last glacial period *in situ*.

CHAPTER 2

MATERIALS AND METHODS

Collections

Populations of *Smelowskia calycina* were sampled from over as wide a range of the distribution of the species as was practical. Localities in which to collect were chosen in part for their accessibility. In addition to pressing plants for study, some were transplanted from each locality into 5-inch pots and removed to growth chamber or greenhouse facilities at the University of Alberta for subsequent use in cytological and limited breeding system studies.

Herbarium Studies

Distributional and morphological studies were made from herbarium specimens as well as from live transplanted material. In the citation of specimens examined (Appendix), the following official abbreviations, adopted in Index Herbariorum (Lanjouw and Stafleu, 1964) are used to designate from which herbarium each specimen was borrowed: University of Alaska (ALA); National Museum of Canada, Ottawa (CAN); Canada Department of Agriculture, Ottawa (DAO); Gray Herbarium of Harvard University (GH); The New York Botanical Garden (NY); Rocky Mountain Herbarium at the University of Wyoming (RM); University of Washington Herbarium (WTU). All other specimens, including those made by the author, are on deposit in the Herbarium of the University of Alberta (ALTA).

Cytology

Most mitotic chromosome counts were made of active growing root

tip cells from plants transplanted from the field and grown in the greenhouse. In a few cases counts were made from germinated seeds.

Root tip tissue to be counted was first fixed in 0.004M 8-hydroxyquinoline (0.116 g in 400 ml H_2O) for three hours at 13°-16°C, following the procedure of Tijo and Levan (1950). The tissue was then washed for five minutes in distilled water and transferred to watch-glasses containing 1% acetic orcein:hydrochloric acid (9:1), heated slightly, and allowed to stain for thirty minutes. Each root tip was then squashed under a coverslip in a drop of 45% acetic acid. The slides were made semi-permanent by ringing the coverslips with a mixture of equal parts of paraffin and balsam resin.

Meiotic figures in pollen mother cells were prepared by first prefixing flower buds in Carnoy's solution for a few seconds, rinsing in water, and fixing for twelve hours at room temperature in Belling's modified Navashin's fluid (Sharma and Sharma, 1965, p. 63), made by mixing equal parts of the following two solutions: Solution A - chromic anhydride (5 g), glacial acetic acid (50 ml), distilled water (320 ml); Solution B - 40% aq formaldehyde (100 ml), distilled water 275 ml). The flower buds were then rinsed thoroughly in distilled water. Anthers were smeared in a drop of 1/2% iron acetocarmine, heated slightly, and squashed under a coverslip. Slides were made semi-permanent using paraffin and balsam resin as mentioned before.

Chromosome counts were made under the oil immersion objective of a Vickers M15c microscope.

Megasporogenesis and megagametogenesis

In an attempt to determine if agamospermy was present in

tetraploid populations of the species, a developmental study of the embryo sac was undertaken to see if any deviations indicative of apomixis did occur. To do this, a recently developed technique for examining embryo sac development using cleared, unsectioned ovules was used following the procedure outlined by Herr (1971; 1973).

Material for the present study was collected from a population of tetraploids on the ridge three miles south of Mountain Park, Alberta (Greene #208, ALTA). Flowers in various stages of pre- and post-anthesis were fixed in the field in FPA_{50} (formalin, propionic acid, 50% ethanol; 5:5:90) for 24 hours at approximately 20°C and then transferred to 70% ethanol at 4°C for storage until use.

To clear the ovules, excised pistils were placed in a solution of 85% lactic acid, chloral hydrate, phenol, clove oil and xylene (2:2:2:2:1 by weight) for 24-48 hours at 20°C. Ovules were then carefully removed from the pistils and placed on specially made slides. (Two size #00 coverslips were secured to a microscope slide about one cm apart with Permount which supported a third coverslip above the ovules to prevent their being squashed.) The ovules were mounted on the slide in a few drops of the clearing fluid and examined under the oil immersion objective of a Zeiss photomicroscope and photographed using Kodak Plus-X pan film. The slides were then discarded.

Guard Cells

A common phenomenon associated with the effect of polyploidy is an increase in cell size (Stebbins, 1971). In a thorough morphological investigation of tetraploid and hexaploid plants of *Galium boreale*, for example, Kliphuis (1973) measured a broad range of morphological characters; the only trait among them which allowed a statistical

separation of cytotypes was guard cell length.

As an indicator of cell size in diploid and tetraploid populations of *Smelowskia calycina*, measurements were made of guard cells. Upper surfaces of dried basal leaves were carefully scraped with a scalpel to remove the dense covering of trichomes. Leaves were then dipped in boiling water until the tissue became limp. A portion of the upper epidermis from the median portion of the leaf blade was peeled off and mounted in water on a microscope slide. The length of guard cells was measured with an eye-piece micrometer on a Vickers M15c microscope.

Trichomes

Increasing use has been made of the scanning electron microscope (SEM) in detecting micromorphological differences between closely related taxa. Rollins (pers. comm.) has used characters of sculpturing and branching of leaf trichomes in assessing relationships in the genus *Lesquerella* with the aid of the scanning electron microscope. In the present study, SEM micrographs were made of basal leaf trichomes of *S. calycina* to detect any differences in surface sculpturing or branching pattern that might be present between diploid and tetraploid populations of the species. Approximately 2 mm^2 sections of dried leaf tissue were mounted on studs, coated with gold and photographed at the SEM facilities of the Department of Entomology at the University of Alberta.

Disc Electrophoresis

With the development of refined techniques of gel electrophoresis, taxonomic studies using soluble plant proteins have become

possible that detect cryptic genetic differences unaccompanied by morphological differences (Hall and Johnson, 1962). The use of seed proteins in systematic studies has been reviewed by Boulter *et al.*, (1966), and Turner (1969) suggests that such protein studies may find their best application in studies of genetic relationships at the infraspecific level.

Discontinuous gel electrophoretic comparisons of soluble seed proteins were made between the diploid and tetraploid populations of *Smelowskia calycina* listed in Table 3. Gels were prepared following the procedure of Davis (1964), outlined below. Following electrophoresis, duplicate gels from each population were developed for the detection of general proteins and the isoenzyme activity of esterases, acid and alkaline phosphatases and catalases.

The acrylamide gels (7 1/2% acrylamide; pore size approximately 100 Å) were prepared using the stock and working solutions listed in Tables 4 and 5, respectively. Equal portions of small pore solutions (1) and (2) were mixed at room temperature and degassed by tapping the mixture in a test tube under a vacuum; this step is necessary to prevent the inhibition of gel polymerization by oxygen that may result in a variable pore size. The solution was carefully pipetted into glass tubes (7 cm long, 5 mm i.d.) stoppered at the bottom. The tubes were filled to within a few mm of the top and covered by a few drops of distilled water allowed to run down the inside of the tube. This was done to prevent miniscus formation and results in the formation of a sharp front that increases band resolution. The filled tubes were then placed over a long-wave ultraviolet lamp to photopolymerize the gels. After about twenty minutes the gels were removed from the UV

Table 3. Seed Collections used in the Disc Electrophoretic Analysis of Proteins

Code Number	2n=	Locality
633f	12	above Branham Lakes, Tobacco Root Mountains, Madison Co., Montana (Greene #633; ALTA)
633g	12	same as 633f except seeds are from plants transplanted to greenhouse facilities to allow seeds to mature
562	22	Mt. Appistoki, Glacier Nat. Park, Montana (Greene #562; ALTA)
646	22	Grave Flats, east of Mountain Park, Alta (Greene #646; ALTA)

Table 4. Stock Solutions for Use in Acrylamide Gel Electrophoresis

A		B	
1N HCl	48 ml	Acrylamide	30 g
TRIS[a]	28.8 g	BIS[c]	0.8 g
TEMED[b]	0.46 ml	H_2O to	100 ml
H_2O to	100 ml		
(pH 8.9-9.1)			

[a] 2-amino-2-(hydroxymethyl)-1,3-propanediol (=tris (hydroxymethyl) aminomethane

[b] N,N,N',N'-tetramethylethylenediamine

[c] N,N'-methylenebisacrylamide

Table 5. Working Solutions for Use in Acrylamide Gel Electrophoresis

Small Pore (1)

1 part A
2 parts B
1 part distilled water
(pH 8.8-9.0)

Small Pore (2)

potassium persulfate		0.14 g
H_2O	to	100 ml

Reservoir Buffer

TRIS		6.0 g
glycine		28.8 g
H_2O	to	1 liter

(pH 8.4-8.6)
(dilute 1:10 with H_2O for use)

light and the water above the gels carefully shaken off. After removing the plugs from the bottoms of the gel tubes, a drop of reservoir buffer was hung from the bottom of each gel, and the tubes were then suspended in the lower reservoir filled with cold buffer. The hanging drops prevent the trapping of air below the gels which might increase the electrical resistence of the gel, thereby causing increased temperatures and poorer protein separation. The upper reservoir was then filled with cold buffer solution to which 1 ml of 0.001% Bromophenol Blue had been added as a marker dye.

Proteins were extracted from ground seed tissue using 10 ml of cold reservoir buffer (Table 5) for each gram of seed tissue. Extraction continued for 24 hours at 4°C before electrophoresing. If the extract was not to be used immediately, it was stored at -8°C until used.

After the gels had been prepared and the electrophoresis apparatus assembled, about 0.1 ml of the seed protein extract was carefully pipetted into the upper end of each gel tube. Electrophoresis was continued for about an hour with a current of 2.5 mA per tube, until the marker dye came to within a few mm of the lower end of the gels. The current was then shut off and the gels removed from the tubes by ringing the gels with a hypodermic needle and water.

Gels to be stained for the detection of proteins were first fixed for an hour in 12.5% trichloroacetic acid (TCA) and then stained in 1% Coomassie Blue diluted 1:20 with 12.5% TCA for 45 minutes. Gels were subsequently rinsed in 10% TCA and stored in 10% TCA in the dark at room temperature for 24 hours to develop. Record was made of blue-staining bands. The gels were then discarded (Chrambach *et al.*,

1967).

Esterase activity of protein bands was determined using Fast Blue RR as the diazonium salt in an α-naphthyl butyrate substrate (Master, 1960). The stain consisted of the following: Fast Blue RR (100 mg); TRIS-HCl buffer (pH 7.0, 10 ml)[1]; 1% α-naphthyl acetate (3 ml)[2]; distilled H_2O (87 ml). Gels were incubated in the solution for 30 minutes at room temperature. Blue-staining bands were recorded and the gels discarded (Brewbaker *et al.*, 1969).

Presence of acid phosphatases was determined by staining with Fast Garnet GBC as the diazonium salt in a Na-α-naphthyl acid phosphate substrate. The stain contained Na-α-naphthyl acid phosphate (100 mg), stain buffer (100 ml)[3] and Fast Garnet GBC (100 mg)[4]. The staining solution was filtered before use. Gels were incubated at 37°C in the stain for 30 minutes. Red-purple bands were recorded and the gels discarded. (Cohen, 1952).

To test for the presence of alkaline phosphatases, gels were stained in a solution containing Na-β-naphthyl phosphate (50 mg), Fast Blue RR (50 mg), $MgSO_4.7\ H_2O$ (123 mg) and Borate buffer (pH 9.7; 100 ml)[5]. Blue bands were recorded and the gels discarded. (Cohen, 1952).

Catalase activity of protein bands was determined by successive staining in the following two solutions: Solution A - 3% H_2O_2 (5 ml),

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[1] 50 ml 1M TRIS + 36 ml 1N HCl.

[2] 1 g α-naphthyl acetate + 50 ml acetone + 50 ml H_2O

[3] 6.8 g Na acetate + 14.8 ml 1N HCl + H_2O to 1 liter; adjust pH to 5.0 using p.1 N HCl.

[4] 4-amino-3,1'-dimethyl azobenzene

[5] 3.74 g Boric acid + 50 ml. 1M NaOH + H_2O to 1 liter; adjust pH to 9.7 with 0.05 M NaOH or 0.06 M Boric Acid.

0.1M Phosphate buffer (pH 7.0; 10 ml)[6], 0.06 M $Na_2SO_2O_3.5\ H_2O$ and H_2O (7 ml); Solution B - 0.09M KI (50 ml) and H_2O (50 ml). Gels were stained in solution A for ten minutes at room temperature, rinsed in distilled water and transferred to solution B to incubate at room temperature until the gels turned dark blue. White bands were recorded before discarding the gels. (Cohen, 1952).

Paper Chromatography

Two-dimensional paper chromatography of plant flavonoids has found increasing use since the pioneering work of Alston and Turner (1963) studying these chemicals to establish interspecific relationship in the genus *Baptisia*. In the present study, flavonoid profiles of diploids and tetraploids were studied using the methods outlined by Mabry *et al.*, (1969).

Basal leaves used in the extraction of the flavonoids were collected in the field and dried in plant presses before being used. Dried leaves from selected populations (Table 6) were pulverized with mortar and pestel and eluted in 10 ml of 80% ethanol per gram of leaf tissue. Approximately 40 drops of the flavonoid extract were spotted on full sheets of Whatman #3MM chromatography paper (46 x 57 cm) in 24 replicates. These were chromatographed descendingly in two dimensions using tert-butyl alcohol:acetic acid:water (TBA in the proportions 3:1:1 and 15% acetic acid for the first and second directions, respectively. Characterization of resolved spots was made using visible and ultra-violet light (3660 Å) on untreated sheets and on sheets developed in 3% aq. ferric chloride and 1%

[6] 13.6 g KH_2PO_4 + 59 ml 1M NaOH + H_2O to 1 liter; adjust pH to 7.0 with 0.1M NaOH.

Table 6. Collections Used in Paper Chromatographic Analysis in Flavonoids

Code Number	2n=	Locality
112	12	Mt. Angeles, Clallam Co., Washington (Greene #112; ALTA)
138	22	5 miles south of Cadomin, Alberta along Grave Flats Road (Greene #138; ALTA)
489	22	Mountain east of Mile 57, Dempster Highway, north of Dawson City, Yukon (Greene #489; ALTA)
562	22	Mt. Appistoki, Glacier Nat. Park, Montana (Greene #562; ALTA)
585	12	St. Mary's Peak, Bitterroot Range, Ravalli Co., Montana (Greene #585; ALTA)
646	22	Grave Flats, east of Mountain Park, Alberta (Greene #646; ALTA)

alcoholic aluminum chloride or on sheets exposed to ammonia fumes.

Compounds present in high concentrations were cut out and eluted in a small amount of spectral grade methanol for spectral identification. Spots that overlapped when initially chromatographed could in same cases be resolved by chromatographing in one dimension using the upper fraction of 1-butanol:acetic acid:water (4:1:5) as the solvent. The spots were cut out, eluted in 80% ethanol, and streaked on half sheets of Whatman #3MM paper to be chromatographed.

Ultra-violet spectral analysis of isolated compounds was performed using a Unicam SP1800 spectrophotometer. Scans of the basic methanol solutions were compared with scans testing the reaction of each unknown with sodium methoxide, aluminum trichloride, aluminum trichloride + hydrochloric acid, sodium acetate, and sodium acetate + boric acid (Mabry *et al.*, 1969, pp. 35-61).

Aglycones of the unknown flavonoids were prepared by refluxing equal volumes of 2N hydrochloric acid and methanol solutions of isolated unknowns for one hour in a reflux condenser. After hydrolysis the aglycone fraction was eluted in ethyl ether and chromatographed in two directions using TBA and acetic acid as outlined above. The aglycones were identified using their Rf values in the solvent systems. The aqueous fraction of each hydrolyzed flavonoid was also chromatographed to identify the sugars present. The solution was spotted on half sheets of Whatman #1 chromatography paper beside standards of glucose, galactose, rhamnose and arabinose using isopropanol:water (4:1). Sheets were then developed using the aniline-

diphenylamine phosphate reagent[7] to stain the sugars. The unknown sugars were identified by comparing their Rf values with those of the standards (Menzies and Seakins, 1969).

[7] made by mixing equal volumes of the following three solutions: A - aniline (5 ml), diphenylamine (5 g), acetic acid (100 ml); B - acetone; C - 85% phosphoric acid (20 ml), water (100 ml).

CHAPTER 3

RESULTS AND DISCUSSION

Cytology

Somatic chromosome counts were determined for the collections listed in Tables 7 and 8. A map showing the distribution of diploid (2n=12) and tetraploid (2n=22) chromosome races in North America based on these data and on the published chromosome counts for the species (Table 2) appears in Map 3. Also indicated on the map are localities where ploidy level has been inferred by guard cell measurements but not confirmed by actual chromosome counts; this information will be discussed in a later section.

These data show that diploids are present from the southern limit of the species' range in Colorado and Utah north to the Swan Range in west-central Montana and to the Olympic Peninsula in Washington. Aneuploids (2n=22) derived from tetraploids occur from Glacier National Park, Montana northward along the main axis of the Rocky Mountains to the vicinity of Mountain Park, Alberta and also occur in Alaska and Yukon. A single true tetraploid (2n-24) was found in a predominantly diploid population[1] on Mount Washington in northwestern Wyoming. This confirms the occasional production of autotetraploid individuals by a diploid population of *S. calycina* and has been documented in a number of other species

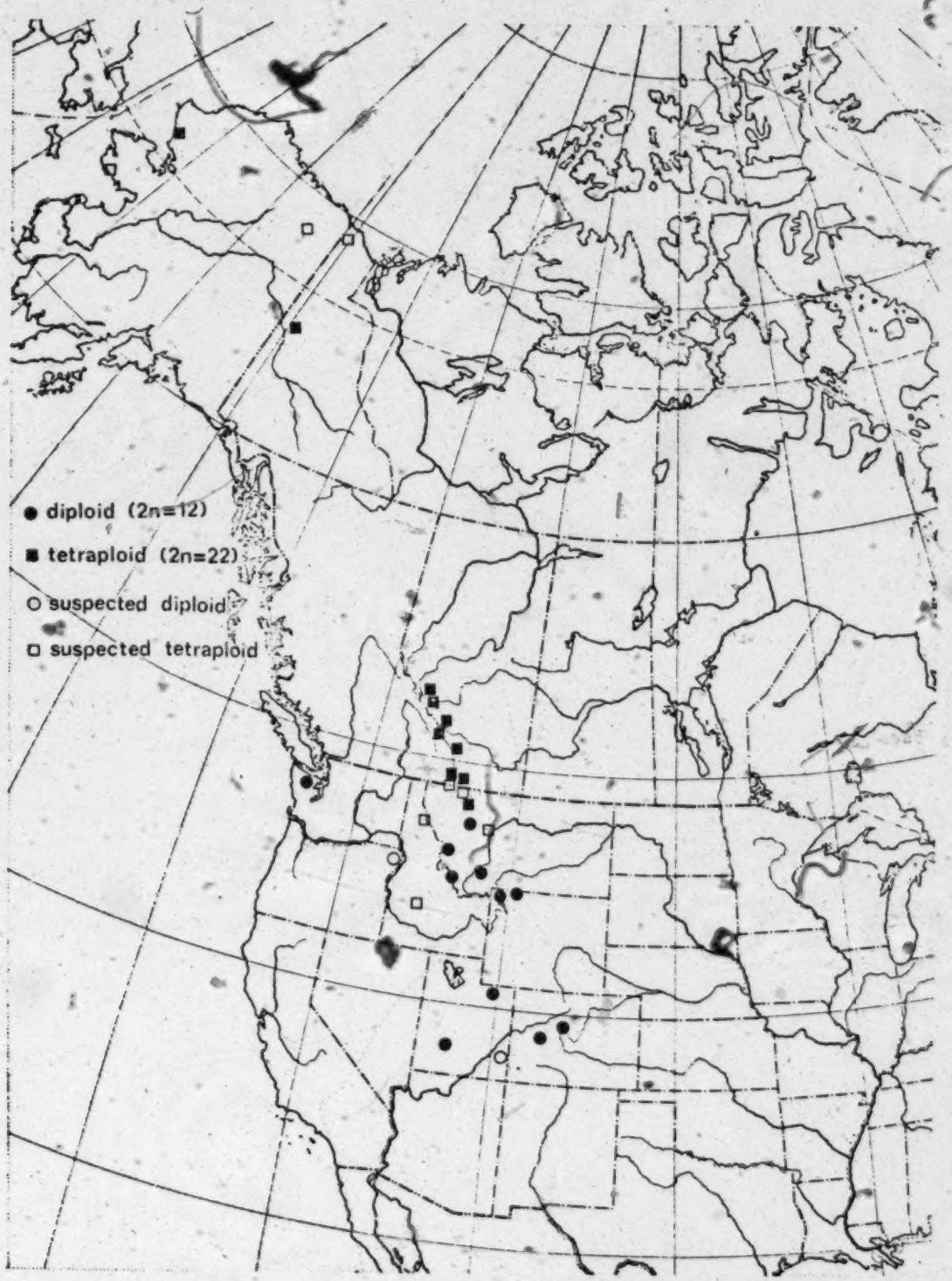
[1]Three other plants from the same population were found to be diploids with 2n=12 chromosomes.

Table 7. Collections on which Diploid (2n=12) chromosome counts are based

Colorado: ridge west of Hoosier Pass, Summit Co., June 20, 1972, C.W. & J.A. Greene #51; Loveland Pass, Clear Creek Co., June 21, 1972, C.W. & J.A. Greene #55. Utah: Bald Mtn., Summit Co., June 24, 1972, C.W. & J.A. Greene #70. Wyoming: Mt. Washburn, Yellowstone Nat. Park, June 27, 1972, C.W. & J.A. Greene #87; Clay Butte, Park Co., June 28, 1972, C.W. and J.A. Greene #103; Beartooth Pass summit, Park Co., June 28, 1972, C.W. & J.A. Greene #109. Montana: above Branham Lakes, Tobacco Root Mts., Madison Co., July 30, 1973, C.W. Greene & M. Archer #633; above Ajax Lake, Anaconda Mts., Beaverhead Co., July 29, 1973, C.W. Greene & M. Archer #624; St. Mary's Peak, Bitterroot Range, Ravalli Co., July 27, 1973, C.W. Greene & M. Archer #585; Smith Creek Pass, Swan Range, Missoula Co., July 25, 1973, C.W. Greene & M. Archer #576. Washington: Mt. Angeles, Olympic Nat. Park. Clallam Co., July 9, 1972, C.W. & J.A. Greene #112. (ALTA)

Table 8. Collections on which tetraploid (2n=22) chromosome counts are based.

Alberta: Upper Carthew Lake, Waterton Lakes Nat. Park, July 14, 1972, C.W. Greene, P. Kuchar #127; above West Castle ski slope, Crowsnest Forest, July 30, 1972, C.W. Green & J. Traquair #191; Highwood Pass summit, July 21, 1972, C.W. & J.A. Greene #153; Snow Creek Pass, Banff Nat. Park, July, 1972, P. Lulman; Grave Flats, Edson Forest, August, 23, 1973, C.W. & J.A. Greene #646; Cheviot Mtn., south of Cadomin, Edson Forest, P.J. Scott. Yukon: mt. east of mile 58, Dempster Hwy., north of Dawson City, Ogilvie Mts., July 10, 1973, C.W. Greene #489. Montana: Mt. Appistoki, Glacier Nat. Park, July 24, 1973, C.W. Greene & M. Archer #562. (ALTA).



Map 3. Distribution of Chromosome Races of *Smelowskia calycina* (Stephan) C.A. Meyer in North America.

of angiosperms as well (Davis and Heywood, 1963; Böcher, 1961).

The small size of mitotic chromosomes in 8- hydroxyquinoline preparations prevented any comparison between diploid and tetraploid karyotypes. Magnification using an oil immersion objective was not sufficiently high to resolve the positions of centromeres or the relative lengths of chromosome arms with any degree of confidence. Hence the derivation of the aneuploids (2n=22) from true tetraploids (2n=24) remains a matter of speculation. The close morphological similarity between diploids and tetraploids (to be discussed later) suggests the origin of the latter by intraspecific hybridization followed by chromosome doubling. The aneuploid condition was probably secondarily derived, either by the loss of a chromosome pair or by translocation of chromosome arms with the subsequent loss of inert centromeres. Loss of a single pair of chromosomes at least in a young autotetraploid, would not deplete the genome, as each pair of chromosomes in the basic genome of the species would be present in duplicate.)

Strict autotetraploids derived from genetically identical parents are not assumed to have any selective advantage over their diploid progenitors (Stebbins, 1950). The success of the tetraploid race of *S. calycina*, then, may be the result of hybridization between genetically different ecotypes. The hybrid vigor inherent in such interecotypic derivatives may create different ecological tolerances that allow the polyploids' adaptation to habitats not suited to either parent (Stebbins, 1971); the result, as in the case of *S. calycina*, may be the allopatric distribution of the original and derived

chromosome races.

Studies of microsporogenesis in tetraploids of the species reveal a cytologically diploid pairing behavior of chromosomes at meiosis, with 11 bivalents present in the few metaphase I figures observed, the migration of 11 univalents to each pole in anaphase I and the regular formation of microspore tetrads after the second meiotic division. In light of the discovery by Riley and Chapman (1958) that chromosome pairing in polyploids can be under genetic control, the bivalent pairing behavior at meiosis gives no indication whether the tetraploids in *S. calycina* are of alloploid or autoploid origin. Nor does the apparent normal meiosis and pollen formation in the stamens necessarily imply a correspondingly normal sequence in megasporogenesis and embryo sac development (Stebbins, 1941, p. 516; Böcher, 1951). The combined effects of tetraploidy and aneuploidy in the 2n=22 chromosome race of *S. calycina* could cause abnormal meiosis resulting in unbalanced gametic products. The adaptive value of apoximis to such a population is obvious and the presence or absence of agamospermy among these tetraploids should be investigated.

The abnormal meiotic behavior and the genetic heterozygosity among many agamospermous species led Ernst (1918, cited by Stebbins, 1950 and Grant, 1971) to suggest hybridization as the cause of apomixis. Similarly, the predominance of polyploidy among known agamospermous taxa (Grant, 1971) suggests a causal relationship between polyploidy and agamospermy. That neither hybridization nor polyploidy is the direct cause of agamospermy is shown by the absence of apomixis in many known hybrid taxa and many polyploid species

(Stebbins, 1950). Agamospermy is now known to be under genetic control (Levan, 1937) and Powers (1945) has proposed a model to show how agamospermy may be promoted by hybridization based on several recessive alleles that are probably involved in the circumvention of the normal sexual process. The observed correlation between polyploidy, hybridization and agamospermy may be explained by the combination of these alleles in a hybrid genome and their fuller expression in polyploids (Stebbins, 1941, p. 526).

Breeding System

Material transplanted from the field and raised in a growth chamber possessed low vigor and was not used in rigidly controlled experiments to study the species' breeding system. Some observations made of these plants while under cultivation, however, may be pertinent in this regard. Over a ten-month period, several diploid and tetraploid plants flowered regularly in the growth chamber under a photoperiod of 17 hours of light and 7 hours of darkness. During this time the plants produced no viable seed with the exception of three artificially cross-pollinated diploid individuals. The chamber housing the plants was free of insect pollinators and cross-pollination due to turbulence in the chamber seems unlikely. The flowers exhibit neither protandry nor protogyny and self-pollinate freely; the introrse anthers located just above the level of the stigma at anthesis allows some pollen to land on stigmas of the same flower. Well-developed nectaries and the aggregation of flowers into large corymbiform heads in the early stages of flowering leave the plants well adapted to insect pollination and field observations in good weather confirmed the frequent visits of dipterans to these flowers. Under natural conditions, then, it seems likely that insects serve as regular self- and cross-pollinators to *S. calycina*, and the species usually sets abundant viable seed in nature.

The observation that some plants raised in the growth chamber flowered regularly but set seed only after cross-pollination suggests the presence of a self-incompatability mechanism. Sporophytic self-incompatability is prevalent in the Cruciferae and was found in

80 of 102 species among 12 tribes of the family investigated by Bateman (1955). The same study showed only a weak correlation between self-incompatability and the perennial habit and that in most species examined the structure of the flower allowed automatic self-pollination. Although *S. calycina* seems to be self-incompatible, some cross-pollinated plants in the growth chamber did not produce any seed and further studies should be undertaken using healthier plants to confirm self-incompatability in the species.

Megasporogenesis and Megagametogenesis

Early in ovule development the integument primordia appear as two rings of actively dividing cells at the base of a crassinucellur nucellus (Figure 1). As development proceeds, the bitegmic ovule assumes a campylotropous form (Figure 2). Uneven growth of the integuments opposite the funiculus directs the micropyle toward the funiculus and at the time of pollination the tip of the outer integument is in contact with the funiculus.

The single archesporial cell is separated from the nucellar epidermis at first by a single layer of cells (Figure 1). It was not determined whether or not a primary parietal cell was cut off from the archesporial initial or if the proliferation of sub-epidermal cells visible in Figure 3 resulted from periclinal division of the nucellar epidermis. The megaspore mother cell appears inactive until after the inner integument has grown to about the length of the nucellus (Figure 3). At this stage, pollen tetrads have already been formed in the anthers. Division of the megaspore mother cell results in a linear tetrad of megaspores of equal size, parallel with the long axis of the nucellus (Figure 4). Soon after their formation, the three micropylar megaspores begin to degenerate as the chalazal megaspore enlarges and becomes vacuolate (Figure 5). This functional megaspore, or megagametophyte mother cell, can be distinguished from the other cells of the nucellus by its large nucleus and prominent nucleolus; one vacuole forms on each side of the nucleus and parallel with the long axis of the nucellus (Figure 6).

Mitotic division of the megagametophyte mother cell first

Figures 1-5

Figure 1. Young ovule showing crassinucellar nucellus with a single archesporial cell. The integuments are just beginning to develop. X 1000. Figure 2. Campylotropous ovule with developing 2-nucleategametophyte. X 380. Figure 3. Megaspore mother cell prior to meiosis. Note inner integument grown to length of the nucellus. X 1160. Figure 4. A linear tetrad of megaspores (arrows) oriented parallel to the long axis of the necellus. X 2120. Figure 5. Enlarging functional megaspore. Arrows indicate three degenerating micropylar megaspores. X 1780.

Key to Labeling: a, antipodal cells; cc, central cytoplasm; e, egg cell; ii, inner integument; mc, megaspore mother cell; n, nucellus, oi, outer integument, pn, polar nucleus, s, synergid cell.

1
2
3
4
5

gives rise to a two-nucleate cell with a single central vacuole (Figure 7) and then to a four-nucleate stage with two nuclei at either end of the much broadened and lengthened embryo sac. By this time the expanding gametophyte has already obliterated the sterile cells in the micropylar end of the nucellus (Figure 8). A third mitotic division results in an eight-nucleate embryo sac. Cytokinesis appears to occur very quickly after this division, cutting off three antipodal cells in the chalazal region of the embryo sac and two hooked synergids and an egg cell at its micropylar end. Polar nuclei migrate from each end to the center of the embryo sac; no wall material separates them (Figure 9). The antipodal cells are ephemeral but can usually be recognized until the two polar nuclei fuse prior to fertilization.

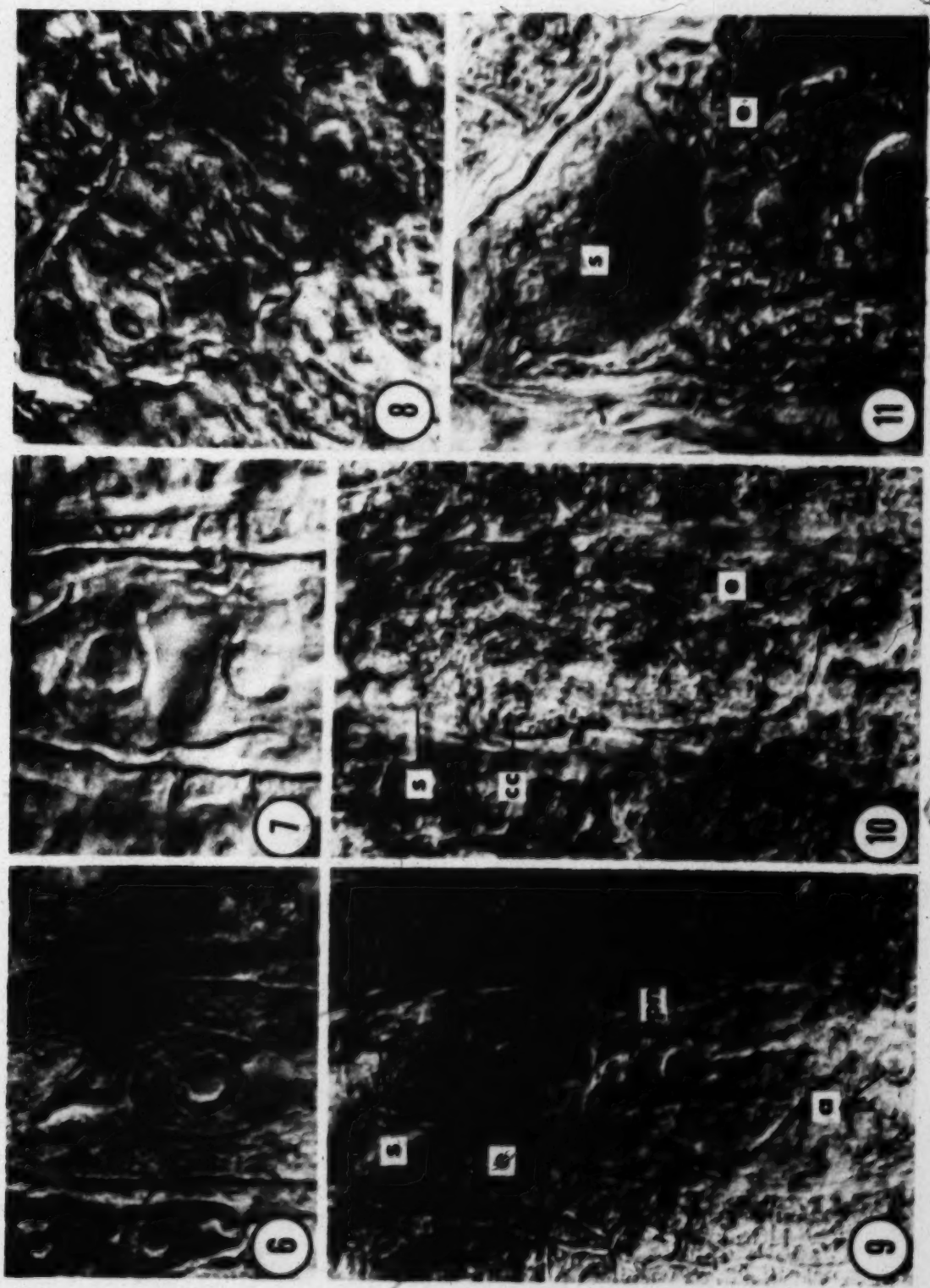
Figure 10 shows the micropylar end of an embryo sac after fusion of the polar nuclei. The hooked shape of the synergids is apparent at this stage; regularly associated with this hook is an extension of the central cytoplasm from the fused polar nuclei. The egg cell is vacuolate with a chalazal nucleus.

Remnants of pollen tubes were regularly observed protruding from micropyles of ovules containing mature, seven-nucleate embryo sacs. The integuments have by this time grown inward toward the funiculus and are almost in contact with it, somewhat distorting the campylotropous nature of the ovule. The course of the pollen tubes could not be traced from the outside opening of the micropyle to the embryo sac. Synergid cells in such ovules have a well-developed filiform apparatus, visible in Figure 11 as a roughened appearance on

Figures 6-11

Figure 6. Gametophyte mother cell. Vacuoles lie on either side of the large nucleus with prominent nucleolus. X 1600. Figure 7. 2-nucleate gametophyte with large central vacuole. X 1500. Figure 8. 4-nucleate gametophyte. Two nuclei lie on either side of a large central vacuole. Note degenerating sterile nucellar cells. X 1340. Figure 9. 8-nucleate, 7-celled gametophyte with polar nuclei and antipodal cells in the plane of focus. X 970. Figure 10. Micropylar end of mature embryo sac after fusion of polar nuclei. Note the extension of central cytoplasm from the fused polar nuclei to the lateral hook of a synergid cell. X 1090. Figure 11. Upper end of embryo sac at about the time of fertilization. Note filiform apparatus on the micropylar end of a synergid cell. Only the degenerated nucellus separates the embryo sac from the inner integument. X 1750.

See page 37 for key to labeling.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the micropylar wall. Plastids become a prominent feature of the central cytoplasm at about the time of fertilization and soon after fusion there is a rapid development of free nuclear endosperm in the chalazal portion of the embryo sac, preceding any apparent division of the zygote. Succeeding stages of endosperm and embryo development were not studied.

Anomalous development occurs within the ovules of all agamospermous angiosperms so far investigated. The absence of a structural gametophyte indicates adventitious embryony, where the embryo develops directly from a somatic cell of the nucellus. Gametophytic apomixis is characterized by the formation of an unreduced gametophyte in one of two ways. Apospory occurs when an integumetary or nucellar cell develops directly into a diploid gametophyte. An aberrant, non-reductional division of a megaspore mother cell giving rise to the embryo sac is termed diplospory. (Stebbins, 1950).

Böcher (1951) first established the occurrence of agamospermy in the Cruciferae by demonstrating diplospory in some diploid and triploid members of the *Arabis holboellii* complex. In these plants, the first meiotic division of the megaspore mother cell is marked by total asyndesis of the chromosomes and may be followed by the formation of a restitution nucleus that undergoes a somatic-like division to form a dyad. One of these unreduced cells divides mitotically to form an embryo sac. The stimulus of a pollen tube entering the embryo sac is necessary for seed formation, but there is no fertilization of either the egg or the fused polar nuclei.

Since Böcher's discovery of apomixis in the Cruciferae, several

other genera in the family have been suspected of being apomictic. Agamospermy has been confirmed in *Draba oligosperma* (Mulligan and Findlay, 1970; Mulligan, 1972) but the exact mechanisms of its embryo sac formation and seed production have yet to be ascertained. Abnormal meiosis in microspore mother cells and the copious production of viable seed in triploids have been used to imply agamospermy in *Erysimum hieraciifolium* (Mulligan and Frankton, 1967), *Parrya arctica* (Mosquin and Hayley, 1966) and in several species of *Arabis* (Mulligan, 1964).

The absence of any agamospermic behavior in the formation of the embryo sac of *S. calycina* discounts the possibility of apomixis in the tetraploid population examined. The production of a linear tetrad of cells from a megaspore mother cell implies that meiosis is normal; subsequent gametophyte formation from the chalazal spore follows the sequence found in a "normal" or "Polygonum" type embryo sac (Maheshawari, 1950). This pattern of embryo sac development is the only one reported for sexually reproducing Cruciferae (Davis, 1966).[2]

Within the family, there is a trend toward progessive sterilization of nucellar tissue. The nucellus of *Smelowskia* is small, with only one archesporial cell and in this regard is similar to the nucelli of *Barbarea vulgaris, Thlapsi arvense* and *Erophila verna*. In contrast to this, the nucellus of *Cardamine praetense* produces multiple archesporial cells, several of which may undergo

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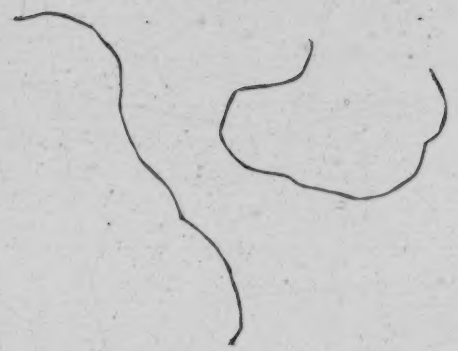
[2]It is reasonable to assume that the same sexual pattern of seed development is present in diploid *S. calycina* as occurs in tetraploid populations of the species.

meiosis before a single megaspore finally gives rise to the embryo sac (Vandendries, 1909). Megagametogenesis is essentially the same throughout the family (Davis, 1966). The campylotropous ovule is a family trait as well and is derived from the anatropous condition (Bocquet, 1959).

Trichomes

Figures 12 to 15 show micrographs of the trichomes of four widely separated populations of diploid and tetraploid races of *Smelowskia calycina*. These micrographs reveal no discernible differences between populations from central Asia, Alaska, Alberta and Wyoming. Moreover, micrographs of basal leaf trichomes of *S. borealis* and *S. ovalis* are indistinguishable from those of *S. calycina*. Thus it appears that characters of trichome sculpturing or branching are not valuable aids to classification within *Smelowskia* or in distinguishing chromosome races in *S. calycina*.

Rollins (1941) found no correlation between trichome size and ploidy level in a study of the indument of *Arabis divaricarpa*. On the other hand, qualitative characters of trichomes have been of great value in the taxonomy of *Arabis* (Rollins, 1941, p. 299) and of many other genera in the Cruciferae (e.g. Hitchcock, 1941; Mulligan, 1972).



Figures 12-15

Figure 12. Basal leaf trichomes of var. *calycina* (2n=12?) from central Asia. Figure 13. Basal leaf trichomes of var. *americana* (2n=12) from northwestern Wyoming. Figure 14. Basal leaf trichomes of var. *integrifolia* (2n=22) from northwestern Alaska. Figure 15. Basal leaf trichomes of var. *americana* (2n=22) from west-central Alberta.

Scale: 1 mm equals 1.43 microns.

12

13

14

15

Guard Cells

A positive correlation exists between ploidy level and guard cell length in *S. calycina*. A significant difference in mean guard cell length between diploids and tetraploids was demonstrated using Student's t test (s=1.29, t=6.87) and a graph showing the range of guard cell length in the two cytotypes is given in Figure 16. As shown in the graph, the range of variation in guard cell length caused considerable overlap between chromosome races. Using an average value for cell length based on 15 measurements per population reduced this overlap to about 10% of the populations studied. The average guard cell length ranged from 16 to 21 microns among known diploids and from 20 to 25 microns among known tetraploids. Although on theoretical grounds there is a significant difference between guard cell lengths in these two groups, it has not always be[redacted] possible to use this information to predict ploidy level in collections from localities where cytological data was not available. In many such collections the guard cell length fell in the range of overlap between the values expected for diploids and tetraploids (20 to 21 microns). Only in cases where the average cell length fell within the range of a single chromosome race was the data used to augment the cytological data presented in Map 3. This information suggests that populations of the species are exclusively tetraploid in Alaska, Yukon and Alberta and that they may be present as far south as the Sawtooth Range in Idaho and the southern part of the Lewis Range in Montana; diploids are found from Washington and west-central Montana to the southern limit of the species' range in the Rocky Mountains.

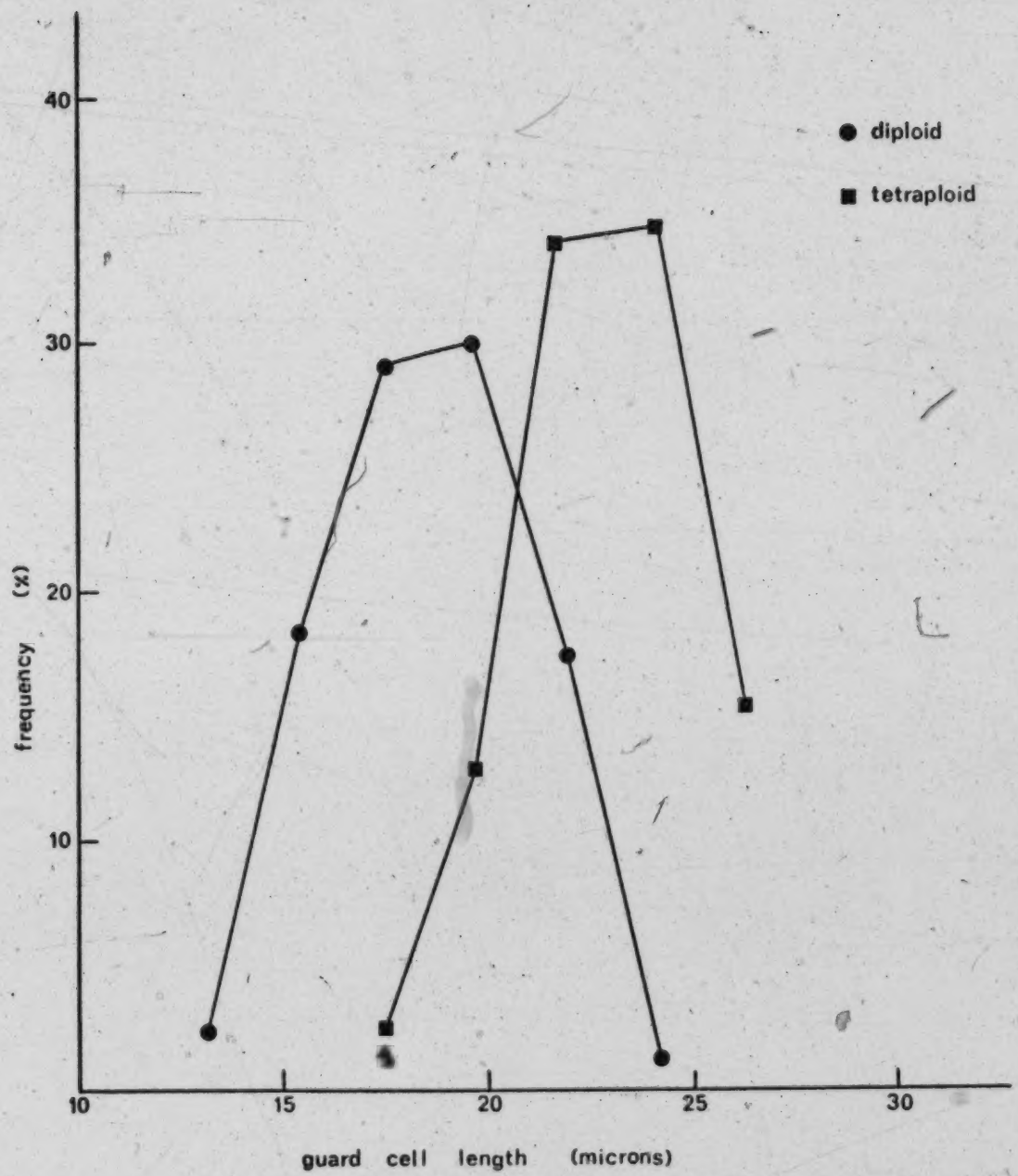


Figure 16. Guard cell length frequencies in *Smelowskia calycina*.

It is not known whether or not the ranges of the two chromosome races are sympatric where they meet in Montana.

Disc Electrophoresis

Banding patterns detected after staining the gels for general proteins, esterases and acid phosphatases are summarized in the zymograms presented in Figures 17, 18 and 19, respectively. In the zymograms, the cathode end represents the origin and the anode end, the position of the marker dye. Broadness of bands is indicative of the intensity of staining of each and stippled areas represent diffuse bands. Although good staining was achieved in the general protein and esterase investigations, only one population of the four studied stained positive for the presence of acid phosphatases. No bands were resolved when stained to detect alkaline phosphatases or catalases.

Rp values were calculated for each band by dividing the distance travelled by the leading edge of the band by the distance travelled by the marker dye. Bands on different gels were considered homologous if their Rp values were within two units of each other, regardless of the intensity of staining of each. A similarity index to express the percentage of similarity between samples was computed using $m/n \times 100$, where m is the number of bands shared by two populations and n is the number of pairs of shared bands plus the number of dissimilar bands present in the two samples. Similarity indices for the general protein and esterase investigations are given in Tables 9 and 10, respectively.

Figure 17. Disc electrophoretic banding patterns for general proteins

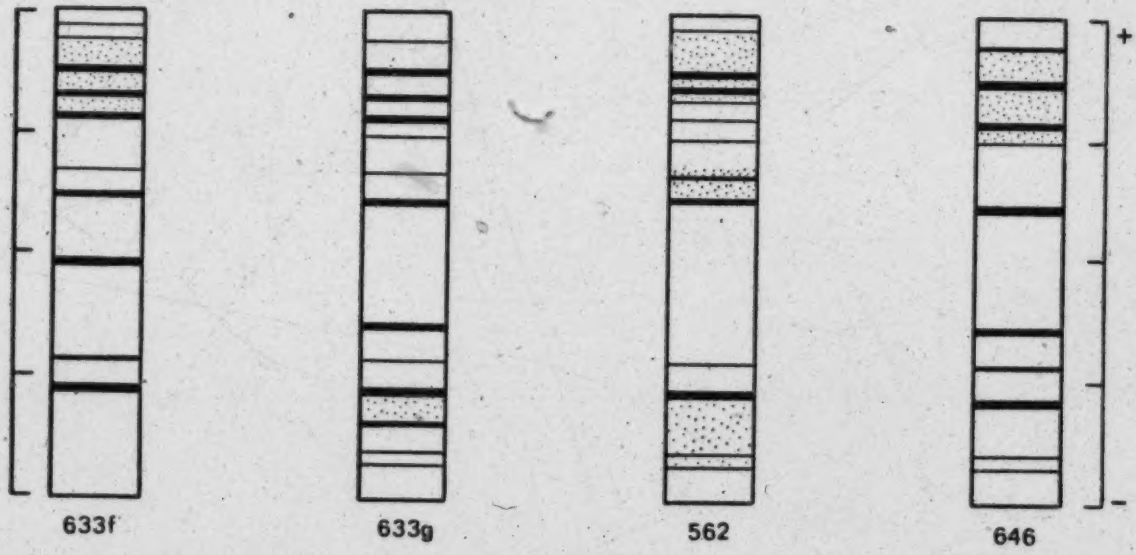


Figure 18. Disc electrophoretic banding patterns for exterases

Table 9. Similarity Indices for General Protein Banding Patterns.

	633f		
633g	60%	633g	
562	31%	50%	562
646	43%	50%	20%

Table 10. Similarity Indices for Esterase Banding Patterns

	633f		
633g	53%	633g	
562	57%	67%	562
646	43%	77%	57%

Although only two general protein bands (Rps .19 and .89) and five esterase bands (Rps .22, .28, .60, .79, and .89) were common to all four samples, an additional seven general protein bands (Rps .06, .12, .15, .33, .51, .67, and .77) and four esterase bands (Rps .67, .74, .83 and .93) were present in at least three of the samples electrophoresed. These bands may represent the typical general proteins and esterases in the species' seed protein make-up. General proteins at Rps .39, .48, .73, .93 and .97 and esterases at Rps .36 and .98 were present in two of the four samples; an additional seven bands were present only once (general proteins at Rps. .26, .56 and .63; esterases at Rps. .16, .48 and .86).

Figure 19. Disc Electrophoretic Banding Pattern for Acid Phosphatases

It might be assumed that populations from within the same chromosome race would have the most similar protein banding patterns, but this does not appear to be the case in *S. calycina*. Populations 562 and 646, the two tetraploids in the study, have the most dissimilar general protein banding patterns (20% similarity) and rank third for esterase similarity (57% similarity). The two diploids samples, 633f and 633g, were from the same population and differed in that the seeds of 633g were matured in the greenhouse on plants transplanted from the field after pollination; the seeds of 633f matured under natural conditions. While their general protein banding patterns rank highest in similarity (60% similarity), the esterase pattern of 633g is more like those of the two tetraploids than it is to 633f. That these two samples did not show a higher degree of similarity suggests either a high degree of intra-populational variation or culturally induced changes in seed protein phenotype in the greenhouse-matured seeds of 633f. Even, however, if one were to disregard these data given for population 633g and to examine only the other three populations whose seeds were matured under natural conditions, no correlation between ploidy level and protein banding pattern would emerge.

Both the general protein and esterase studies reveal a surprisingly low degree of similarity between populations, with similarity indices ranging from 20% to 60% for general proteins and from 43% to 77% for esterases. Intraspecific studies of isoenzyme patterns in other species show considerably higher indices of similarity. For example, Vaughan and Gordon (1973), studying seed

esterases in *Brassica juncea*, demonstrated a 64% to 100% similarity in banding patterns between Indian and oriental races of the species; most samples were at least 91% similar. Pollen protein banding patterns between populations of *Betula populifolia* in New Jersey averaged 86.5% similar for esterases, 43.0% similar for general proteins separated in acid gels and 71.2% similar for general proteins separated in basic gels (Payne and Fairbrothers, 1973). In view of the history of geographic isolation of populations of *Smelowskia calycina*, the regional variability evidenced by low percentage similarities may indicate the fixation of distinct genotypes in different parts of the species' range after long periods of genetic isolation.

Although the small sample size prevents establishing the range of variation of seed protein banding patterns in the species, the initial data suggest that no correlation exists between protein banding pattern and ploidy level. It should be mentioned that these data represent a study of qualitative measurements of proteins and not a quantitative one, whereas the "dosage effect" of a duplicate genome in the tetraploids may produce quantitative differences not revealed in this study. In initial studies of the effect of a duplicate genome on seed protein banding patterns, Murray *et al.*, (1970) found no detectable quantitative differences between seed proteins of diploids and colchicine-induced tetraploids of *Avena pilosa*, but these workers cautioned that the situation may be different in natural autotetraploids undergoing subsequent evolution. Comparisons of seed protein profiles of natural diploids

and autotetraploids in *Phlox* by Levin and Schaal (1970) revealed neither quantitative nor qualitative differences.

Paper Chromatography

A master chromatogram showing all the spots resolved in the paper chromatographic study of flavonoids in two diploid and four tetraploid populations of *S. calycina* is presented in Figure 20. Table 11 shows the presence or absence of each of these compounds in each population sampled. Rf values and color reactions of the spots are given in Table 12; Table 13 gives ultraviolet spectral absorbence data for compounds present in higher concentrations.

Of the twenty-six spots observed among the populations studied, only three were of universal occurrence (spots 1, 9 and 14). An additional five compounds were present in at least three of the six populations (spots 2, 10, 11, 13 and 17); twelve of the spots occurred only once and were generally small spots present in low concentrations. None of the spots occurred exclusively in all the chromatograms of a given chromosome race, but spot #2 may be a possible marker for tetraploids, as it was present in three out of four tetraploids examined.

The flavonoid extract from a single population of *Smelowskia borealis*[3] was chromatographed for comparison with the flavonoid profile of *S. calycina*. Its spot pattern consisted of nine spots, six of which corresponded in Rf values and color reactions to spots 1, 2, 7, 9, 11 and 14 of the *S. calycina* profile. These spots may represent typical leaf flavonoid components of the genus as a whole.

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[3]Collected on the peak south of Mt. Klotz, northwest of Dawson City, Yukon (Greene #329; ALTA).

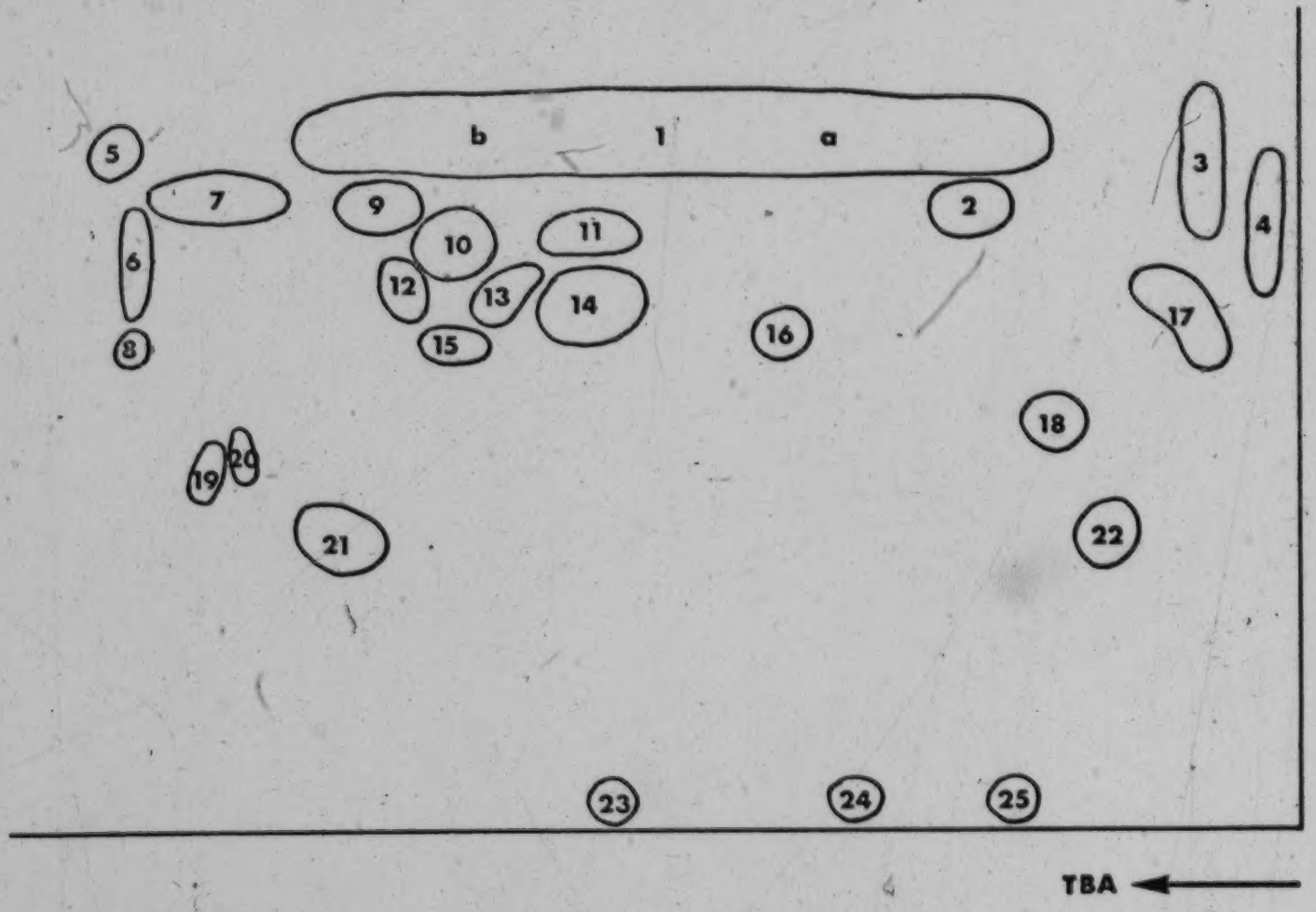


Figure 20. Master Chromatogram of Flavonoids in *Smelowskia calycina*.

Table 11. Fluorescent Compounds Present in Populations Studied

	Population					
Spot No.	112a	138b	489b	562b	585a	646b
1a	+	+	+	+	+	+
1b	+	+	+	+	+	+
2	-	-	+	+	-	+
3	+	-	-	-	-	-
4	+	-	-	-	-	-
5	+	-	-	-	-	-
6	-	-	+	-	-	-
7	+	-	+	-	-	-
8	-	-	-	-	-	+
9	+	+	+	+	+	+
10	+	+	-	+	+	+
11	+	+	-	+	+	+
12	+	-	-	-	-	-
13	-	-	+	-	+	+
14	+	+	+	+	+	+
15	+	-	-	-	-	-
16	-	-	-	-	-	+
17	-	-	+	+	+	+
18	-	-	-	+	+	-
19	-	-	-	-	+	-
20	-	-	-	-	+	-
21	-	-	-	+	+	-
22	-	-	+	-	-	-
23	-	-	-	-	+	-
24	+	-	-	-	-	-
25	-	-	-	+		-

a, 2n=12

b, 2n=22

Table 12. Color Reactions of Spots

Spot No.	Visible	UV Untreated	NH_3	$AlCl_3$	FeCl
1	br	p	y	y	bl
2	br	bl	-	-	-
3	-	p	-	y	-
4	-	p	-	y	-
5	-	bl	y	-	-
6	-	bl	-	-	-
7	-	bl	-	-	-
8	-	y	-	-	-
9	-	bl	-	-	-
10	-	p	-	y	-
11	-	bl	-	-	-
12	-	bl	-	-	-
13	-	bl	-	y	-
14	br	p	y	y	bl
15	-	d	-	-	-
16	-	bl	-	-	-
17	-	p	y	y	-
18	-	p	-	y	-
19	-	g	-	-	-
20	-	bl-g	-	-	-
21	-	p	-	-	-
22	-	p	-	-	-
23	-	y	-	-	-
24	-	bl	-	-	-
25	-	l	-	-	-

Key to colors: bl-blue, br-brown, d-dark, g-gray, l-light,p-purple, y-yellow

Table 13. Spectral Data for Isolated Flavonoids

Population and Spot No.	Rf TBA	Rf HOAc	Color UV/NH_3	MeOH BI	MeOH BII	NaOMe BI	NaOAc BII	$AlCl_3$ BI	+HCl BI	H_3BO_3 BI	Aglycone Rf/TBA	Sugar
112 #1a	.25	.85	y	355	257	+70	0	+45	-45	+20		arabinose
489 #1a	.45	.85	y	354	252	+46	0	+82	-38	+12	.57	arabinose
562 #1a	.60	.85	y	356	249	+46	0	+96	-38	+16	.59	arabinose
112 #1b	.35	.85	y	340	245	+48	0	- 8	- 6	+ 8		arabinose
138 #1b*	.40	.85	y	355	248	+46	0	+46	-40	0	.56	arabinose
489 #1b	.58	.85	y	343	248	+57	0	+97	-40	+11	.58	arabinose
562 #1b	.65	.85	y	342	248	+54	0	+78	-40	+ 8	.57	arabinose
646 #1b*	.70	.85	y	354	250	+42	0	+46	-40	0	.56	arabinose
112 #14	.30	.68	y	360	258	+64	-3	+45	-44	+16		arabinose
138 #14*	.35	.60	y	360	272	+45					.58	arabinose
562 #14	.45	.65	y	360	255	+68	-5	+40	0	+20	.61	arabinose
585 #14	.55	.65	y	360	256	+66	-4	+80	-36	+14	.57	arabinose
112 #10	.50	.75		348	264	0	0	+42	0	0		arabinose
562 #10*	.63	.73		354	250	+46	+4	0	0	0		
585 #10	.74	.70		352	250	+48		+48	-40	0		
112 #11*	.35	.75		350	250	+46		+5			.59	arabinose
562 #23*	.55	.05		328	240						.58	arabinose
562 #18*	.22	.62	y	289	253						.64	arabinose
585 #17*	.23	.61	y	365	250						.69	arabinose
112 #9*	.55	.82		322	245						.69	arabinose
585 #9*	.80	.75		320	252	+80	-3	0	0	+14		

Table 13 (cont'd)

Population and Spot No.	Rf TBA	Rf HOAc	Color UV/NH_3	MeOH BI	MeOH BII	NaOMe BI	NaOAc BII	$AlCl_3$ BI	+HCl BI	H_3BO_3 BI	Aglycone Rf/TBA	Sugar
585 #18*	.30	.52	y	360	250						.58	arabinose
562 #9*	.69	.78		310	250	+80		+20	-20	0		
562 #21*	.60	.40		368	252							

*Poor concentration

Explanation of Table: MeOH - major absorption peaks (Band 1 and Band II) of basic methanol scan; NaOMe - shift of band I relative to MeOH scan after addition of sodium methoxide; NaOAc - shift of Band II relative to MeOH scan after addition of sodium acetate; $AlCl_3$ - shift in Band I relative to MeOH scan after addition of aluminum trichloride; +HCl - shift in Band I relative to $AlCl_3$ scan after addition of hydrochloric acid to $AlCl_3$ solution; H_3BO_3 - shift in Band I relative to MeOH scan after addition of boric acid to NaOAc solution; (+) = bathochromatic shift of peak absorption wavelengths; (-) = hypsochromatic shift of peak absorption wavelength; (y) = yellow; all wavelength espressed in nm; for procedural details, see Mabry *et al.*, 1969.

In all populations of *S. calycina* examined, as well as in the single population of *S. borealis* sampled, spot 1 was the most prominent and present in the highest concentration. This spot was really a complex of several compounds that separated poorly in the TBA solvent (perhaps due to their presence in such high concentrations). Two compounds isolated from this spot complex were identified using the ultraviolet spects, and their chemical structures were given in Figures 21 and 22. The second of these, quercetin 3-O diarabinoside 7-O arabinoside, moved slightly slower than the first, 3-O arabinoside 7-O diarabinoside, in the BAW solvent. An additional one or two flavonols making up spot 1 moved slightly slower than these in BAW but could not be sufficiently isolated and purified to be identified, but are assumed to be compounds with structures similar to those given in Figures 21 and 22. A third flavonol glycoside, spot 14, was tentatively identified as quercetin 3-O triarabinoside (Figure 23), but was not available in high enough a concentration to allow its positive identification; its movement in the solvents indicates a less highly glycosylated flavonol derivative than the compound in spot 1.

The absence of the simple mono- and diglycoside derivatives of quercetin in *S. calycina* seems unusual in light of the predominance of such highly glycosylated quercetin derivatives; one would expect to detect some of their precursor flavonols in association with them. That this is not the case may indicate their synthesis pathways are

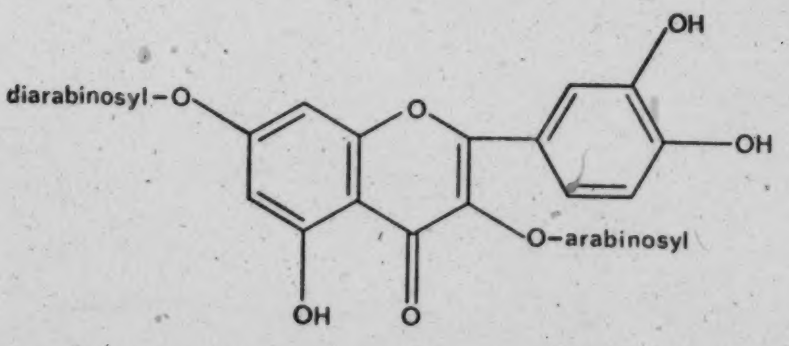


Figure 21. Quercetin 3-0 arabinoside 7-0 diarabinoside

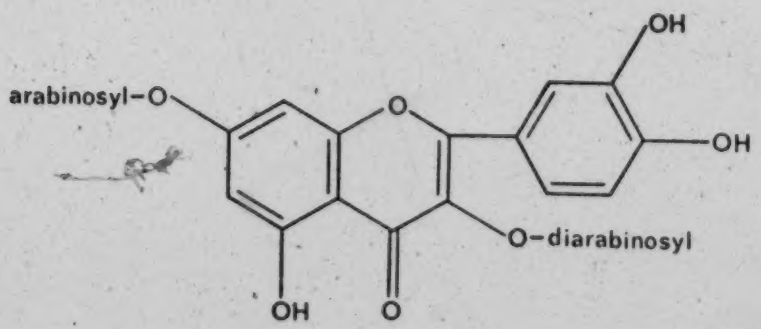


Figure 22. Quercetin 3-0 diarabinoside 7-0 arabinoside

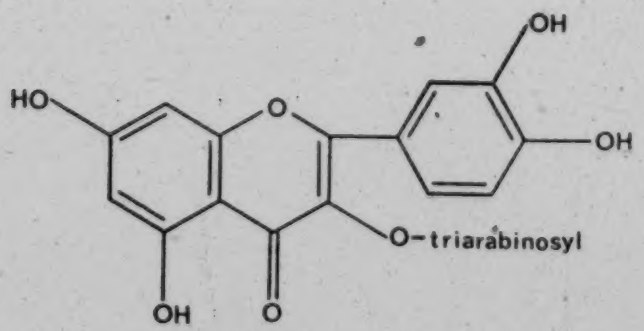


Figure 23. Quercetin 3-0 triarabinoside

not through the simpler flavonol glycosides but through corresponding glycosides of another class of flavonoids or that the sugar molecules may be bound to parts of the flavonol molecule before its formation (Ribereau-Gayon, 1972, pp. 209-214).

A similar situation is apparent in other cruciferous genera; in *Brassica* and *Sinapis* species simple 3-monoglucosides of kaempferol and isorhamnetin are of considerably less frequent occurrence than their more highly glycosylated derivatives. While flavonols of the latter type occurred in almost all the taxa of these two genera examined by Durkee and Harborne (1973), 3-O glycosides were present in only three of the twenty-four species or cultivars examined.

The presence of flavonol glycosides in itself is characteristic in the Cruciferae, but surveys in other genera of the family, e.g. that of Durkee and Harborne (1973), revealed their presence in only low concentrations. The striking feature of the *Smelowskia* flavonoid profile, in contrast, is the presence of these highly glycosylated flavonols in high concentrations.

Of the five monosaccharides associated with phenolic compounds (D-glucose, D-galactose, D-xylose, L-rhamnose and L-arabinose), L-arabinose is of the least frequent occurrence (Harborne, 1964) and flavonoid arabinosides in the Cruciferae have not been reported in the literature. The detection of only arabinose in the sugar fraction of hydroloyzed extracts of nine flavonoids isolated from *S. calycina* (see Table 13) seems interesting in this light. As the survey of flavonoid glycosides in cruciferous genera proceeds, the ability of some of them to synthesize arabinose may become a valuable taxonomic criterion in descriminating generic and tribal affinities in the family. Durkee and Harborne (1973), too, suggested the possible

importance of the types of flavonol glycosides in future chemosystematic studies within the Cruciferae.

Percentage similarity between populations' spot patterns was calculated the same as was done in the disc electrophoretic study, using the statistic m/n; these values appear in Table 14. Percentage similarity ranges from 28% to 60%, values comparable to those observed in the seed protein studies (Tables 9 and 10). The data suggest no correlation between flavonoid profile and ploidy level but seem to indicate a regional variability of pattern components. Population 112 from the Olympic Peninsula and population 489 from Yukon show the lowest average percentage similarity to the rest of the populations sampled, 34% and 39%, respectively. The other four populations were collected within 400 miles of each other along the main axis of the Rockies from Montana to Alberta and had average percentage similarity to all other populations of from 44% to 51%. The highest percentage similarity, 60%, was between a diploid (585) and a tetraploid (562) population collected less than 100 miles from one another. The lowest observed similarity was between Olympic Peninsula and Yukon populations.

The comparison of flavonoid constituents of diploid and tetraploid populations of *S. calycina*, in summary, demonstrates the common occurrence in all of a few flavonols in high concentrations. The occurrence of a similar flavonoid profile in *S. borealis* suggests these chemicals may be common to the genus as a whole. In addition, many compounds present in lower concentrations are of more restricted occurrence and indicate a high degree of genetic diversity among

Table 14. Similarity Indices for Flavonoid Profiles.

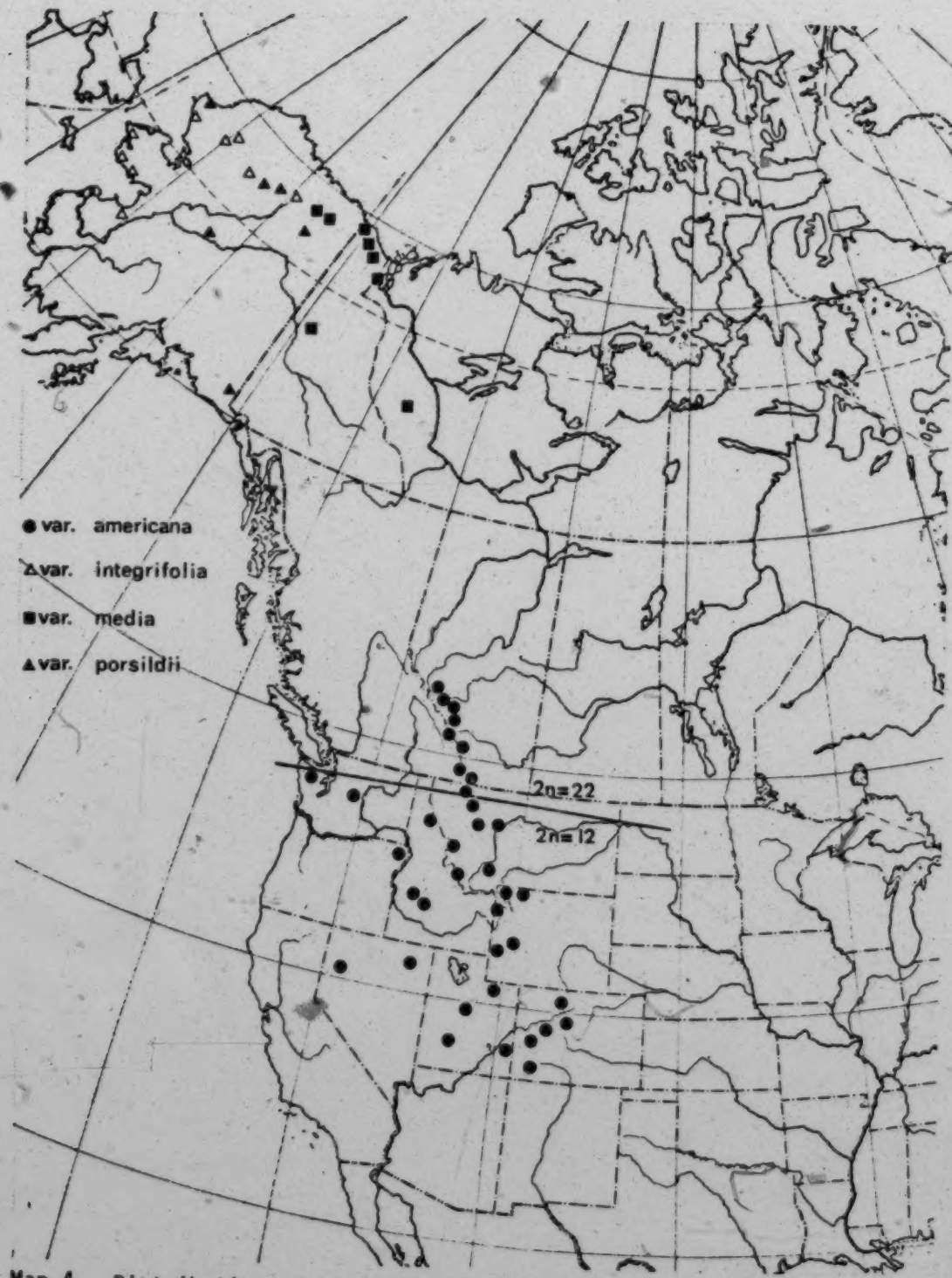
	112				
138	46%	138			
489	28%	33%	489		
562	33%	55%	50%	562	
585	30%	46%	35%	60%	585
646	33%	55%	50%	57%	50%

populations of the species. Discontinuities in the flavonoid profiles of populations are apparently independent of the ploidy level of populations but indicate more extreme differences between populations the farther apart they grow from one another.

Morphology

Studies of morphological variation among populations of *Smelowskia calycina* for the most part corroborate the varietal classification of the species in North America proposed by Drury and Rollins (1952). Especially in regard to basal leaf shape, var. *americana*, var. *porsildii* and var. *integrifolia* are readily distinguishable; var. *americana* has many-lobed, pinnately dissected leaves, var. *porsildii* has narrow, long-petiolate basal leaves and var. *integrifolia* has short-petiolate, oval or obovate leaves (see Figures 24-26). The populations of the De Long Mountains in the westernmost part of the Brooks Range, referred by Drury and Rollins to var. *integrifolia* (1952, p. 104) are probably better placed in var. *porsildii*. These plants, as well as those of the Ogotoruk Creek-Cape Thompson area, typically have narrow leaves with petioles longer than the leaf blades. Although Drury and Rollins stressed the broad angle of divergence of pedicels as a key character in separating var. *integrifolia* from var. *porsildii*, there is considerable variation in this character and separating the taxa base on leaf shape is more dependable. (The constancy of these characters was established in controlled environment studies.) Map 4 shows the distribution of the varieties of *S. calycina* in North America.

Variety *media* has a more variable morphology than shown by the few specimens available to Drury and Rollins as revealed in the examination of recently available collections from northeastern Alaska, Yukon and the North west Territories. Drury and Rollins' "typical" var. *media* has mostly 3-7 lobed and some entire basal



Map 4. Distribution of the presently recognized varieties of *Smelowskia calycina* in North America.

Figure 24. *Smelowskia calycina* var. *americana* (Regel & Herder) Drury & Rollins

Smelowskia calycina (Stephan) C. A. Meyer
var. americana (Regel & Herder) Drury
& Rollins

Alberta: 2½ yards south of Highwood
Pass summit, mile 91.1, 100 feet east
of road.

Coll. C. W. Greene # 153 July 21, 1972
& J. A. Greene

Figure 25. *Smelowskia calycina* var. *porsildii* Drury & Rollins

(see discussion re. placement of Cape Thompson-Ogotoruk Creek populations in var. *integrifolia* (Seeman) Rollins)

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Salicornia
CALYCINA
VAR. INTERMEDIA
2n = 22
OG. CREEK
N.W. ALASKA

UNIVERSITY OF ALASKA

Plants of Ogotoruk Creek, N.W. Alaska

...ornia fruticosa (Linnaeus) Goodman
...var. intermedia (Lange) Hultén

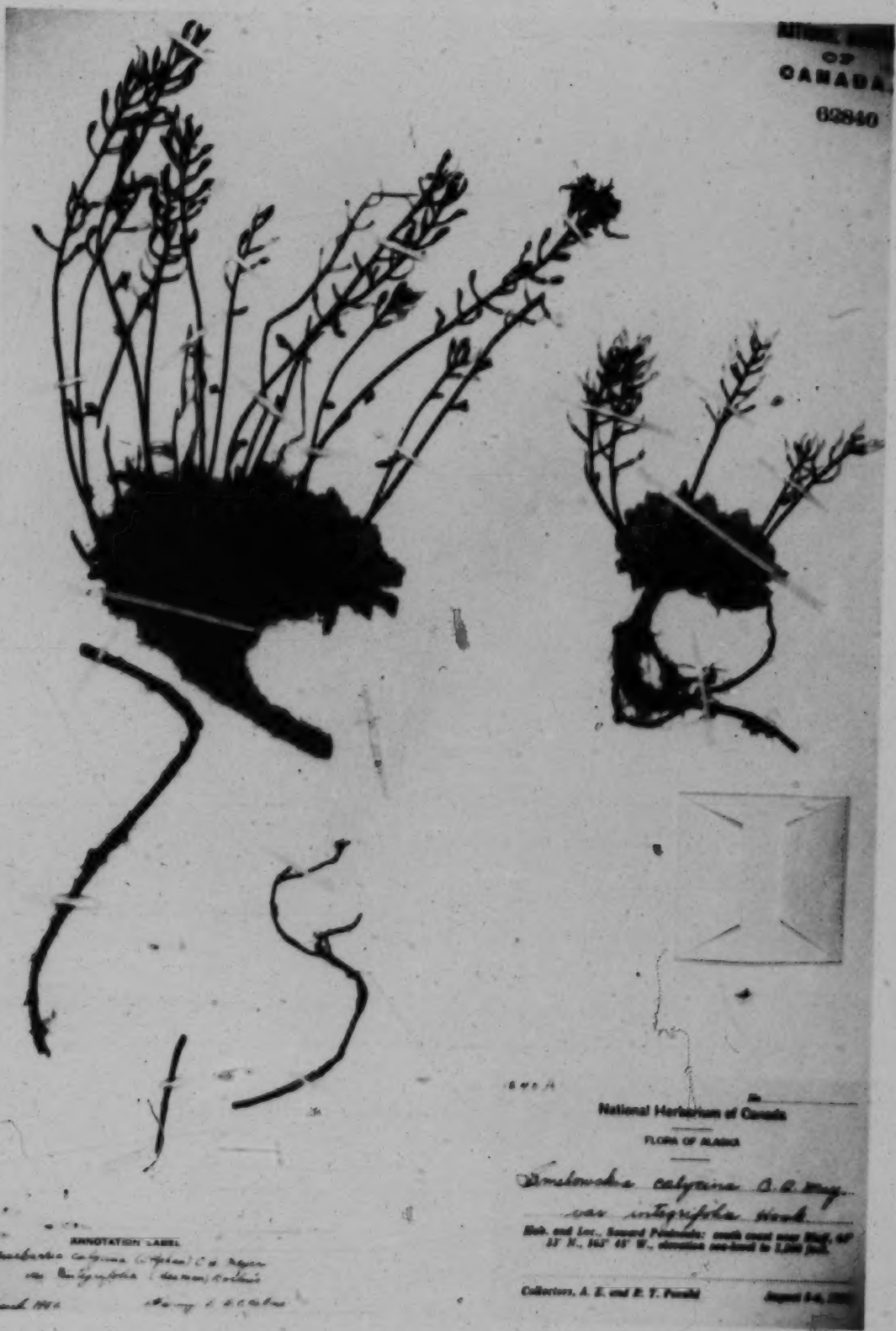
...ddish slopes - Dryadetum ...

...ck of dunelike ridge north of creek.

Chromosome number 2n = 22

J. G. Packer, Calif. 1971. 2 July 1968.

Figure 26. *Smelowskia calycina* var. *integrifolia* (Seeman) Rollins


OF
CANADA
62840
National Herbarium of Canada
FLORA OF ALASKA
Arnebia calycina A. A. Mey.
var integrifolia Hook
Collectors, A. E. and E. T. Porsild
ANNOTATION LABEL

leaves, but some populations examined by this author, in addition to having this "typical" type, include individuals with only ovate, entire leaves suggesting var. *integrifolia*. The predominance in others of more narrow, entire leaves than lobed leaves suggests var. *porsildii*. A case in point is a collection made by this author from a population of var. *media* which includes an array of plants encompassing most of the range of variation found among all the arctic varieties (Figure 27). This example emphasizes the point made by Drury and Rollins that intergradations between these arctic varieties are to be expected; however, the named varieties serve as useful points of reference in treating the broad range of morphological variation encountered.

The present study has shown that var. *media* is tetraploid and is therefore not the phylogenetic intermediate between the Asiatic and American diploids, as was suspected by Drury and Rollins. However, its broad range of variation may be evidence that it is the phylogenetic link between tetraploid populations of var. *americana* and the other arctic varieties. This is in partial agreement with the observation made by Drury and Rollins (1952, p. 91) that "the accentuation of various trends [in var. *media*] could have produced the other taxa of the *calycina* complex in North America."

Of special interest has been the search for morphological markers to distinguish diploids from tetraploids in *S. calycina*. The arctic varieties, all of which are here assumed to be tetraploid, are easily distinguished from the Rocky Mountain material based on the predominance in the former of entire to few-lobed basal leaves.

Figure 27. *Smelowskia calycina* var. *media* Drury & Rollins

Arctic basin collection (Greph.) C.A.Meyer
var. scrip Gray & Hollins

Arctic Wtn.: Just below summit of
pass east of mile 56, Dempster Hwy.,
north of Dawson City, Yukon; 66°27'N,
13°1o'w; alt. 5000-6000 feet.

Open scree slope; southern exposure.

Coll.: C. W. Greene #409 July10, 1973

Among the latter, however, no clear discontinuities were found to exist between the diploids and tetraploids (with the exception of guard cell size already discussed). Although there is regional variability within var. *americana*, this is not correlated with ploidy level. This type of variability permits the trained eye to recognize minor variation patterns characteristic of a certain part of the range of the variety. It was this type of minor variation in leaf lobation and pubescence that led Rydberg to name three species, *S. americana, S. linearilobа* and *S. lobata*, from the Rocky Mountain populations of *S. calycina* (Rydberg, 1902, 1904, 1912).

The only potentially useful character of value in separating diploids from tetraploids in var. *americana* is the tendency of tetraploids to produce entire basal leaves in addition to dissected ones. Individuals from within the range of the diploids occasionally produce a very few entire basal leaves, but in many Alberta and Montana tetraploid populations examined some individuals were found with almost equal numbers of dissected and entire basal leaves. In this way the tetraploid populations of var. *americana* approach the form of typical var. *media*; this may be indicative of the phylogenetic affinity of the two based on cytological data.

Stebbins (1971, p. 139) has observed that the range of variation is less in intraspecific tetraploids than in their diploid progenitors, due to the stabilizing effect of tetrasomic inheritance. The pattern of variation among arctic tetraploids of *S. calycina* is, however, in contradiction to this. Drury and Rollins (1952, p. 95) have reasoned that the survival in this region of small, isolated

homogeneous populations has resulted in the persistence of several discrete morphological varieties. A similar type of survival probably existed in the southern part of the species range in the Rocky Mountains. Why, then, is the regional variation present there not nearly as great as among populations of Alaska and Yukon?

In polyploids of ancient origin the accumulation of such a range of variation is to be expected, but the evidence suggests that the origin of the tetraploid race of *S. calycina* is more recent, probably associated with the drastic climatic changes accompanying the Pleistocene glaciations. Even the variation among tetraploid complexes considerably older than the expected age of tetraploid *S. calycina* supports the conservative rate of genetic change in tetraploids relative to their diploid counterparts (Stebbins, 1971, p. 148). It seems clear, then, that some additional factor has played a role in establishing such a diversity among the arctic populations of the species in question. Although the diveristy of *S. calycina* in the arctic might be explained by its mode of survival during the Pleistocene, some genetic mechanism may well be involved. A possible mechanism allowing increased variability in tetraploids may be the introgression of the tetraploid with a now-extinct race of the species in the general area of Alaska and Yukon. Marks (1966) has demonstrated unidirectional introgressive hybridization from diploids to their tetraploid derivatives via unreduced gametes; in a system such as this, the phenotypic variability of the tetraploid can be increased by gene exchange with a diploid.

Distribution

Each of the chromosome races of *Smelowskia calycina* has a disjunct distribution. Diploids are disjunct between central Asia and the southern Rocky Mountains and tetraploids are disjunct between northwestern North America and the central Rocky Mountains. In both cases the disjunctions involve territories glaciated during the Pleistocene period and can best be explained in relation to the successive advances and retreats of the Pleistocene ice sheets.

The presence of diploids south of the maximum extent of Pleistocene ice in the mountains of central Asia and western North America suggests their former continuous distribution in suitable habitats between these mountain regions. Hultén (1937) proposed the survival of this taxon in these southern glacial refugia and in a third area as well, viz. unglaciated Alaska and Yukon (see Introduction). Hultén's basis for assuming the survival of the same (diploid) species in this large refugial area, however, is entirely plausible, as the distributions of numerous species listed by him attest. The cytological evidence and the inference of ploidy level through guard cell measurements presented here confirms only the presence of tetraploid populations in Alaska and Yukon, but the persistence of diploids of the species there cannot be conclusively discounted until a much more thorough cytological examination of arctic populations has been completed. At any rate, the diploids found in the Rocky Mountains from Colorado, Utah and Nevada north to western Montana and in the Cascade and Olympic Ranges of Oregon and Washington are here considered remnants of a once continuous distribution of

diploids to the mountains of central Asia in intra- or pre-Pleistocene times. Their presence in unglaciated or only locally glaciated regions is significant; the extinction of biotypes of the species which accompanied the advance of Pleistocene ice over part of the species' former range appears to have so reduced the ecological amplitude of the diploids that they were incapable of reoccupying glaciated habitats in their former range subsequent to the retreat of the ice.

Duplication of a genome is largely an irreversible process (Stebbins, 1971, p. 156) and in a polyploid series one can safely assume the origin of higher ploidy levels from lower ones. The independent formation of polyploids in different parts of a species' range is possible, but in the case of *S. calycina*, the tetraploid race is here assumed to have been secondarily modified by the aneuploid loss of two chromosomes (from 2n=24 to 2n=22). The independent recurrence of both chromosome duplication and the aneuploid loss of two chromosomes in separate tetraploid populations is probably so small that one can assume the disjunct 2n=22 populations of the species to have a single origin.

The disjunct distributional pattern of tetraploid populations of *S. calycina* suggests a formerly continuous distribution from the northern limit of the range of the diploids in the Rocky Mountains north to the arctic and possibly west across the Bering Land bridge into Siberia[4].

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[4]Zhukova (in Yurtsev and Zhukova, 1972) has reported chromosome counts of 2n=24 for two populations of *Smelowskia porsildii* (Drury & Rollins) Yurtsev in the Chukotsk region of Siberia. Apparently the pattern of variation among these populations is identical to that found in var. *porsildii* in Alaska (Yurtsev, 1969).

Relictual populations have apparently survived the most recent glaciation in northwestern Montana and isolated localities in the mountains of Alberta, as well as in the vast refugium of Alaska and Yukon.

The allopatric ranges of the two chromosome races of *S. calycina* and the coincidence of the boundary between them with the approximate maximum extent of Pleistocene ice (Richmond, 1965; Richmond *et al.*, 1965; Map 5) suggests a causal relationship between their ranges and the glacial history of the region. For instance, Manton (1934) showed the distribution of diploids of *Biscutella laevigata* to be restricted to unglaciated areas around the European Alps and that the tetraploids are widespread in adjacent glaciated regions. Similarly, Randhawa and Beamish (1972) have shown the restriction of diploids of *Saxifraga ferruginea* to habitats south of the ice margin in western North America, while polyploids of the species occur in areas glaciated in late Pleistocene time. The conclusion reached in both these studies is that the diploids represent relicts from before the most recent glaciation and that polyploids derived from them migrated to occupy glaciated habitats laid bare by the retreat of the most recent glaciers.

Many factors, among them latitude, elevation, extreme climate, glaciation and type of habitat, have been cited by various authors to explain the success of polyploids relative to their diploid progenitors; good discussions of the several observed correlations between these and polyploidy appear in Stebbins (1971) and Grant (1971). Most successful polyploids are probably of inter-specific or interecotypic origin (Stebbins, 1950, p. 301) and their mode of

Map 5. Maximum extent of Wisconsin glaciation in North America (based on Can. Geol. Survey Map No. 1257A).

inheritance (based on four alleles instead of two) maintains their genetic heterozygosity. Their inherent heterosis, then, may give them a selective advantage over diploids in exploiting new ecological niches.

To explain the correlation between polyploidy and glaciation, Stebbins (1971, pp. 185-188) envisions the proximity of differently adapted ecotypes of a species or group of related species in adjacent habitats modified by the climatic and edaphic changes accompanying glaciation. Repeated hybridization between these would result in the establishment of new ecotypes, some of them polyploids, in diverse periglacial habitats. Some of these would be adapted to colonize the territories left bare as the glacier receded. Johnson and Packer (1965) have presented evidence to suggest that polyploids may have a selective advantage over diploids in colonizing such terrain. Their study of plant communities in northwestern Alaska shows that the frequency of polyploids is highest in habitats having the most extreme edaphic environments, characterized by fine soil texture, high soil moisture, low soil temperature and a high degree of disturbance. Although their research was in an area left unglaciated in Pleistocene and recent times, comparable extremes in the edaphic environment accompany glaciation.

The periglacial origin of the tetraploid race of *Smelowskia calycina* appears to have followed a pattern similar to the one outlined above. Its origin could have been in any one of the refugia where diploids of the species survived the early Pleistocene glaciations, viz. in central Asia, Beringia, or south of the ice in

western North America. The 2n=24 populations of *S. calycina* var. *porsildii* in the Chukotsk region of Siberia could be a remnant of the chromosome race from which the 2n=22 populations are derived (see footnote [4]). Alternatively, the close morphological similarity between diploids and tetraploids in adjacent regions of Montana and Alberta suggests the polyploid's origin in this area[5]. The previously mentioned coincidence of the line separating their ranges with that of the maximum extent of Pleistocene ice may also support this view. The present distribution of the tetraploids indicates that they colonized glaciated regions following an early or penultimate Pleistocene glaciation and had their widest distribution prior to the advance of the Wisconsin ice sheets. Their present local distribution in areas apparently not covered during the most recent glaciation indicates their perglacial survival there. They were, like the diploids, apparently unable to remigrate into glaciated habitats subsequent to the retreat of the Wisconsin glaciers.

Map 5, depicting the maximum extent of the Wisconsin glaciation in western North America, shows that the present range of *Smelowskia calycina* was ice-free during the Wisconsin except in the Canadian Rocky Mountains (Map 6). The contention, then, that the occurrence of the species in these Alberta localities represents survival *in situ* throughout the Wisconsin glaciation and not post-glacial stepwise or long-distance dispersal, demands special

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[5] These populations differ from those of central Asian *S. calycina* in having a caducous calyx, but in all other respects are almost identical to the Asian material, with which the type of the species is associated (Rollins, 1938; Drury and Rollins, 1952).

Map 6. Distribution of *Smelowskia calycina* (Stephan) C.A. Meyer in the Canadian Rocky Mountains.

consideration.

Although the Canadian Rocky Mountains were for the most part completely covered by the Cordilleran ice mass in Wisconsin time, the higher peaks are known to have protruded above the ice as nunataks (Heusser, 1956; Williams & Bayrock, 1966). In addition, the lower precipitation east of the continental divide resulted in a thinner ice cover than was present to the west and may have allowed ice-free areas to exist along the eastern front ranges in Alberta during the Wisconsin (Packer and Vitt, 1974).

Smelowskia calycina is of widespread occurrence in the Waterton Lakes region of Alberta, but its distribution north of there is sporadic. The species has been collected on Mount Rundle, Quartz Hill and the Snow Creek Pass region of Banff National Park and in the following localities close to the eastern edge of the Canadian Rockies: above the West Castle River, North Kootenay Mountain; Hailstone Butte; Ram Mountain; Grave Flats and in the vicinity of Mountain Park, Alberta (Map 6).

Alternative to its persistence in these localities throughout the last glaciation is the possibility of the post-glacial dissemination of *S. calycina* from territory either north or south of the glaciated region. Except for its occurrence in the localities listed above, the species is apparently absent elsewhere in the mountains of Alberta. The broad disjunctions in its distribution within the province tend to discount the possibility of a stepwise migration of the species from unglaciated areas in post-glacial time. The seeds of *Smelowskia* are wingless, barbless and have no other

adaptations to facilitate their dispersal. Even if the chance long-distance dispersal of their seeds were accepted, this is not a reasonable explanation for the species' establishment primarily in the front ranges. The species has no edaphic specialization, as witnessed by its almost ubiquitous presence on most of the higher peaks in the southern Rocky Mountains and on a variety of rock types. The species' absence in apparently suitable alpine habitats over most of the Canadian Rocky Mountains, then, tends to discount the importance of long-distance dispersal in explaining the present distribution of the species.

Smelowskia calycina is apparently well adapted to survival in bleak alpine habitats, judging from its abundance on exposed summits of the higher peaks of the southern Rocky Mountains. It seems, then, to be a likely candidate for the proposed survival of vascular plants in isolated nunatak areas throughout the last glaciation. That this is the only reasonable explanation to account for the species' present distribution pattern in the mountains of Alberta has been corroborated in a recent paper by Packer and Vitt (1974), who have presented both geological and botanical evidence in support of a Wisconsin period plant refugium in the vicinity of Mountain Park, Alberta. The coincident distribution of several vascular plants and bryophytes of disjunct distribution in this area and in several other *Smelowskia* localities as well, is strong evidence in favor of their perglacial survival *in situ*; no other reasonable explanation for the coincident distribution of this plant assemblage is plausible in light of the evidence presented

by Packer and Vitt (1974).

CHAPTER 4

CONCLUSIONS

The present study has demonstrated the allopatric distribution of two distinct chromosome races of *Smelowskia calycina* in North America. The history of geographical isolation of populations during the Quaternary Period has led to the establishment of minor morphological and chemical variation patterns, neither of which reflects the karyological discontinuity between diploids and tetraploids. The infraspecific classifications proposed for *Smelowskia calycina* (Drury and Rollins, 1952; Hultén 1945, 1968) have been based on the morphological discontinuities and obscure the natural relationships between populations of each chromosome race.

A primary role of biosystematics is to define the natural limits of reproductively isolated breeding populations, i.e. biological species. Polyploidy represents the abrupt creation of an almost complete barrier to genetic exchange between diploids and the polyploids derived from them. Stressing the importance of this barrier to genetic exchange, some proponents of the biological species concept have suggested that each chromosome race of polyploid complexes be given taxonomic recognition at the specific level (Löve, 1951; Valentine and Löve, 1958). This poses a problem for diploid and autopolyploid populations that, like *Smelowskia calycina*, are morphologically indistinguishable from one another; the evolution of distinct, reproductively isolated chromosome races has been

unaccompanied by any apparent genetic changes affecting their phenotypes. In the interest of a practical taxonomy, then, it seems best to retain both chromosome races of *Smelowskia calycina* within the same species, stressing their genetic continuity and not their reproductive discontinuity. Nevertheless, each race has had a distinct history since their evolutionary divergence sometime during the Pleistocene and it seems most appropriate to recognize each at the subspecific level. It is here proposed that the tetraploid populations of *S. calycina* be included in subsp. *integrifolia* (Seeman) Hultén. This subspecies, then, would include the three arctic varieties recognized by Drury and Rollins (1952), var. *integrifolia*, var. *porsildii* and var. *media*, as well as those populations of var. *americana* with the 2n=22 chromosome complement. As the type of var. *americana* is Borgeau's collection made on Palliser's expedition in the Bow River region of Alberta (specimen in NY), it is probably tetraploid. The type material of *S. calycina* from the Altai region of central Asia is probably diploid (see Yurtsev and Zhukova, 1972) and the diploid populations of the species in the southern Rocky Mountains should be placed with the Asian populations in subsp. *calycina*. In sympathy with the views of Drury and Rollins (1952) that the Rocky Mountain population of *S. calycina* is varietally distinct from the Asian populations, this author proposes the name var. *montana* for the Rocky Mountain diploids formerly included in var. *americana*. As was mentioned in the discussion (p. 27), tetraploid individuals (2n=24) may occasionally be found among diploid populations.

Synopsis of *Smelowskia calycina* in North America[1]

Basal leaves entire or 3- to 5-lobed; cauline leaves entire or 3- to 5-lobed; chromosome number 2n=22.
- Basal leaves obovate to oval, entire or shallowly 3-lobed at the tip; petioles shorter than the leaf blades; plants of the Seward Peninsula area . . subsp. *integrifolia* var. *integrifolia*
- Basal leaves linear to spatulate, mostly entire but occasionally pinnately 3- to 5-lobed; petioles longer than leaf blades; plants of the Brooks Range . . subsp. *integrifolia* var. *porsildii*

Basal leaves mostly pinnately divided, only occasionally entire; cauline leaves pinnately lobed; chromosome number 2n=12 or 22.
- Basal leaves pinnately 3-to 7-lobed, with some leaves usually entire; plants of eastern Brooks Range and Mackenzie Ranges; chromosome number 2n=22 . . . . . subsp. *integrifolia* var. *media*
- Basal leaves pinnately 7- to many-lobed, entire basal leaves uncommon; plants of the Rocky Mountains.
  - Plants of the southern Rocky Mountains north to Washington and northwestern Montana; chromosome number 2n=12 . . . . . subsp. *calycina* var. *montana*
  - Plants of the central Rocky Mountains from the northern Lewis Range in Montana north to west-central Alberta; chromosome number 2n=22 . . subsp. *integrifolia* var. *americana*

Smelowskia calycina (Stephan) C. A. Meyer subsp. *calycina* var. *montana* var. nov.

folia basala plerumque pinnatisecta, segmenta 5-11, oblonga vel cuneata, folia rarissime integerrima; cellulae accessoriae (13)-16-21-(24) u longae; numerus chromosomorum 2n=12.

basal leaves pinnately divided into 5-11 oblong to cuneate segments, rarely entire; basal leaf guard cells (13)-16-21-(24) u long; chromosome number 2n=12.

Holotype: Clay Butte, 10,000 ft., Park Co., Wyoming, June 28, 1972, *C. W. & J. A. Greene 103* (ALTA).

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[1] New names and combinations to be validly published at a later date.

Distribution: Colorado, Utah and Nevada north to Washington, Idaho (?) and Montana to the Swan and Mission Ranges.

Smelowskia calycina subsp. *integrifolia* (Seeman) Hultén var. *integrifolia*

basal leaves oval to obovate, entire or shallowly 3-lobed at the tip; petioles shorter than leaf blades; chromosome number 2n=22.
Distribution: coastal western Alaska in the vicinity of the Seward Peninsula.

Smelowskia calycina subsp. *integrifolia* var. *porsildii* (Drury & Rollins) Hultén

basal leaves linear to spatulate, entire or pinnately 3- to 5-lobed; petioles longer than leaf blades; chromosome number 2n=22.
Distribution: Cape Thompson area east to the eastern end of the Brooks Range; southeastern Alaska.

Smelowskia calycina subsp. *integrifolia* var. *media* (Drury & Rollins) comb. nov.

Smelowskia calycina var. *media* Drury & Rollins

basal leaves pinnately 3- to 7-lobed, oblong to oval, with some leaves usually entire; cauline leaves pinnately lobed; chromosome number 2n=22.
Distribution: eastern Brooks Range, Richardson and Mackenzie Mountains.

Smelowskia calycina subsp. *integrifolia* var. *americana* (Regel & Herder) comb. nov.

Hutchinsia calycina β *americana* Regel & Herder

basal leaves pinnately divided into 5-11 cuneate to oblong segments

or occasionally entire; cauline leaves pinnately divided; chromosome number 2n=22.

Distribution: northern Lewis Range in Montana north to west-central Alberta.

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APPENDIX

REPRESENTATIVE SPECIMENS

Smelowskia calycina (Stephan) C. A. Meyer
subsp. *calycina* var. *montana* var. nov.

HOLOTYPE: Clay Butte, 10000 ft., Park Co., Wyoming, *C.W. & J.A. Greene 103* (ALTA).

MONTANA: McDonald's Peak, Mission Range, *Mr. & Mrs. Canby 32* (CAN;NY); Deerlodge Co., top of peak south of Storm Lake, Anaconda Mountains, *C. L. Hitchcock & C. V. Muhlick 14792* (NY); Missoula Co., summit of Smith Creek Pass, east of Condon, Swan Range, *C. W. Greene & M. Archer 576* (ALTA); Ravalli Co., summit of St. Mary's Peak, Bitterroot Mountains, *C. W. Greene & M. Archer 585* (ALTA); Beaverhead Co., in cirque above Ajax Lake, Anaconda Range, *C. W. Greene & M. Archer 624* (ALTA); Madison Co., on peak southeast of Branham Lakes, Tobacco Root Mountains, east of Sheridan, *C. W. Greene & M. Archer 633* (ALTA). IDAHO: Lemhi Co., Liberty Mt., in Lemhi Range, Gilmore, *J. H. Christ & W. W. Ward 14858* (NY). WASHINGTON: Chelan Co., open talus slopes, Mt. Stuart, *J. W. Thompson 7679* (NY); Chelan Co., Bryan Butte, *G. H. Ward 18* (WTU); Jefferson Co., alpine ridges in Marmot Pass, 7500 ft., *J. W. Thompson 9939* (NY; WTU); Clallam Co., Mt. Angeles, Olympic Mts., *J. W. Thompson 8389* (GH; WTU); Mt. Angeles, *C. W. & J. A. Greene 112* (ALTA); Hurricane Ridge, Olympic Mts., *J. W. Thompson 10591* (RM; WTU); OREGON: Wallowa Co., Aneroid Lake Area, above 4 mile mark, Wallowa Mts., *A. R. Kruckeberg 2235* (CAN; NY; RM; WTU). WYOMING: The Thunderer, Yellowstone Nat. Park, *A. & E. Nelson 5821* (NY;RM); Mt. Washburn, Yellowstone Nat. Park, *C. W. & J. A. Greene 87* (ALTA); Park Co., Clay Butte, ½ mile west of Beartooth Butte, 10000 ft., *R. C. Rollins & C. L. Porter 51270* (GH; NY; RM; WTU); Park Co., 1 mile east of summit of Beartooth Pass, *C. W. & J. A. Greene 110* (ALTA); Park Co., about 3 miles southwest of summit of Cooke City-Red Lodge Highway, *C. L. Hitchcock 23908* (NY); Grand Teton Nat. Park, head of south fork, Cascade Creek, west of Jenny Lake, *F. W. Pennell 24223* (GH); Fremont Co., 3 miles northwest of Brooks Lake, Wind River Range, 10700 ft., *R. W. Scott 852* (NY; RM); Fremont Co., Horse Ridge, east of Dinwoody Creek, Garnett Peak area, Wind River Range, *F. X. Jozwik 363* (GH);

Sublette Co., summit of Saltlick Mt., northeast of Kendall, *E. B. & L. B. Payson 2952* (RM); Lincoln Co., Mt. Wagner, southeast of Smoot, *E. B. Payson & G. M. Armstrong 3747* (RM). COLORADO: Clear Creek Co., ¼ mile east of Loveland Pass summit, south of road, *C. W. & J. A. Greene 50* (ALTA); Douglas Mt., 12000 ft., Georgetown, *M. E. Jones 447* (NY; holotype of *S. linearifloba* Rydb.); Park Co., southwest slope of Hoosier Ridge, 1½ miles east of Hoosier Pass, *R. C. Rollins & W. A. Weber 5161* (GH; RM); Hoosier Ridge, *J. G. Packer 4231* (ALTA); Park Co., ridge east of Hoosier Pass, 12000 ft., *C. W. & J. A. Greene 51* (ALTA); Pitkin Co., summit of Independence Pass, *C. W. & J. A. Greene 54* (ALTA); Gunnison Co., Rustlers Gulch, above Gothic, *J. A. Ewan 11833* (WTU); San Juan Co., southeast slope of Engineer Mt., 12500 ft., *R. C. Rollins 51140* (GH; RM); La Plata Mts., Cumberland Basin, 12000 ft., *C. F. Baker 580* (NY); Conejos Co., Conejos Peak, 13000 ft., *H. D. Harrington 1797* (RM). UTAH: Bald Mt., Wahsatch Mts., 12500 ft., *M. E. Jones 1936* (NY); Summit Co., Bald Mt., 11800 ft., *C. W. & J. A. Greene 70*(ALTA); Salt Lake Co., Big Cottonwood Canyon, 10000 ft., *A. O. Garrett 1301* (GH); Utah Co., near summit of Mt. Timpanogos, 11950 ft., *B. Maguire 17530* (NY); Grand Co., La Sal Mts., 11500 ft., *E. B. & L. B. Payson 3982* (RM; WTU); Mountains north of Bullion Creek, near Marysvale, *P. A. Rydberg & E. C. Carlton 7092* (NY). NEVADA: locality uncertain, *S. Watson 100* (NY); Humboldt Nat. Forest, Shell Creek Range, at base of South Shell Peak in North Fork of Berry Creek, *N. H. Holmgren 2236* (NY).

subsp. *integrifolia* (Seeman) Hultén
var. *americana* (Regel & Herder) comb. nov.

ALBERTA: northern Rocky Mts., Palliser's expedition, *E. Borgeau* (NY; holotype of *S. lobata* Rydb.; syntype of *Hutchinsia calycina americana* Regel & Herder); Mountain Park, back of "Miner's Roof", *M. O. Malte & W. R. Watson 1980* (CAN); south of road past Mountain Park, above Cardinal River, *J. G. Packer 2750* (ALTA); 5½ miles south of Cadomin, west of Grave Flats Road, *C. W. & J. A. Greene 138* (ALTA); Ridge east of Grave Flats Road summit, 3 miles south of Mountain Park, *C. W. Greene & S. Wiatr 208* (ALTA); Ram Mountain, Rocky Mountain House, *G. Rankin & L. Tretiak 782* (ALTA); Snow Creek

Pass, 30 miles north of Banff, *J. A. Calder 23954* (DOA); Upper slopes of Mt. Rundle, 7500 ft., *W. C. McCalla 2274* (NY); North summit of Quartz Hill, Sunshine region, Banff Nat. Park, *C. W. Greene & J. Traquair 198* (ALTA); Elbow River, Rocky Mts., 49°40'N, *J. Macoun 18172* (GH); Highwood Pass, south of Kananaskis, *E. H. Moss 10730* (ALTA; CAN); Highwood Pass summit, *C. W. & J. A. Greene 153* (ALTA); East summit of North Kootenai Pass, *G. M. Dawson* (CAN; GH); Hailstone Butte, 6 miles southwest of Willow Creek, *M. G. Dumais & R. Kempinsky 7311* (ALTA); North end of ridge above West Castle Ski Slope, southwest of Pincher Creek, *C. W. Greene & J. Traquair 191* (ALTA); Waterton Lakes Nat. Park, east of outlet of Upper Carthew Lake, *C. W. Greene & P. Kuchar 127* (ALTA); Waterton Lakes Nat. Park, Sofa Mt., *J. Kuijt 5148* (ALTA; CAN); Waterton Lakes Nat. Park, south-facing slope of Mt. Lineham, *A. J. Breitung 16409* (GH; NY). BRITISH COLUMBIA: Flathead area, head of McClatchie Creek, *D. Pyke 40* (DOA); Flathead Road, mile 19, *M. Bell & J. Davidson 119* (DOA); Northwest of Twin Lakes Peak, 49°08'N, 114°10'W, *R. L. Taylor & D. H. Ferguson 2349* (NY). MONTANA: Park Co., just east of Piegan Pass, 7500 ft., *C. W. Greene & J. Traquair 189* (ALTA); Glacier Nat. Park, Logan Pass, *B. Maguire 1559* (GH); Glacier Nat. Park, Mt. Appistoki, slope above Appistoki Falls, *C. W. Greene & M. Archer 562* (ALTA); Powell Co., top of Gordon Mt., 6 miles south of Big Prairie Ranger Station, Flathead Nat. Forest, *C. L. Hitchcock 18878* (DOA; GH; RM; WTU)*. IDAHO: Shoshone Co., summit of Stevens Peak, *J. B. Leiberg 1480* (NY; RM); Custer Co., north slope of Castle Peak, White Cloud Range, Challis Nat. Forest, *C. L. Hitchcock & C. V. Muhlick 10862* (NY; RM; WTU)*; Custer Co., Leatherman Pass, Lost River Mts., west fork of Pahsimeroi River, *C. L. Hitchcock & C. V. Muhlick 11156* (CAN; RM)*; Blaine Co., Soldier Mts., *MacBride & Payson 2889* (GH; NY; RM)*.

subsp. *integrifolia* (Seeman) Hultén
var. *integrifolia*

ALASKA: Anvil Hill, Nome, *A. E. & R. T. Porsild 1358* (ALA; CAN);

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*placed in var. *americana* solely on the basis of guard cell measurements.

Anvil Ridge, Nome, *W. B. Miller 128-c* (ALA); Cape Nome, *E. LePage 23826* (DOA); Seward Peninsula, south coast near Bluff, *A. E. & R. T. Porsild 1240* (CAN; ALTA); Norton Sound, low volcanic hills at Quqertariaq, 1000 ft., *A. E. & R. T. Porsild 1050* (CAN); Ogotoruk Creek, Crowbill ridge, 600 ft., *J. G. Packer 1783* (ALTA).

subsp. *integrifolia* (Seeman) Hultén
var. *porsildii* (Drury & Rollins) Hultén

ALASKA: Cape Thompson, 68°05'N, 165°40'W, 2½ miles southeast of Base, *R. C. & M. Wood 532* (CAN); Sea cliffs ca. ½ mile north of Cape Thompson, 68°06'N, 166°W, *L. H. Benson* (ALA, acc. no. 28710); Ogotoruk Creek, 68°06'N, 165°46'W, Crowbill Ridge, *J. G. Packer 1973* (ALTA); Bering Strait District, Ogotoruk Creek drainage and vicinity, *R. Johnson & B. Neiland 43* (ALA); Televirak Hills, *J. G. Packer 1827* (ALTA); Kokrines Mts., north side of divide toward Melozitna River, 65°20'N, 154°30'W, *A. E. & R. T. Porsild 741* (CAN); West of Pitmega River, about 5.5 km south-southeast of Cape Sabine, *S. G. Shelter & K. J. Stone 3150* (CAN); Chandler Lake, 68°12'N, 152°41'W, *K. L. Wiggins 13739* (WTU); Anaktuvuk Pass, 68°17'N, 151°25'W, 3000 ft., *L. A. Spetzman 1894* (CAN); Signal Mtn., 10 miles northwest of Old John Lake, Brooks Range, *L. H. Jordal 3710* (CAN: paratype of var. *porsildii* Drury & Rollins); Headwaters of the White River, mountains in vicinity of terminus of Russell Glacier, 61°42'N, 141°45'W, *D. F. Murray 2200* (ALA).

subsp. *integrifolia* (Seeman) Hultén
var. *media* (Drury & Rollins) comb. nov.

ALASKA: Lake Schrader, 69°25'N, 145°W, 4000 ft., *L. A. Spetzman 653* (CAN; isotype of var. *media* Drury & Rollins). YUKON: opposite Mt. Conybeare, 8 miles southeast of Komakuk Beach DEW Line site, 69°36'N, 140°15'W, 300 ft., *J. A. Parmelee 2869* (DOA); Small lake north of Trout Lake, near Babbage River at edge of British Mts., 68°51'N, 138°42'W, *J. A. Calder 34367* (NY; WTU); Mt. Sedgewick, British Mts., 68°53'N, 139°06'W, *J. A. Calder 34454* (DOA); East slope of Richardson Mts., west of Mackenzie River Delta, approx. 68°N, 136°W, *A. E. Porsild 6821* (CAN); Canoe Lake, east slope of Richardson Mts., 25

miles west of Aklavik, 68°13'N, 135°54'W, *W. J. Cody & S. Johansson 12876* (DOA); Ogilvie Mts., mountain east of mile 57-58, 6000 ft., *R. T. Porsild 255* (GH; WTU); mountain east of mile 57-58, Dempster Highway, north of Dawson City, *C. W. Greene 489* (ALTA). NORTHWEST TERRITORY: Mackenzie Mts., Redstone River Region, 63°35'N, 127°W, 5000 ft., *E. Kvale & K. Haggard 145* (DOA).